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ICoMST
Responsible meat production
70th International Congress of Meat Science and Technology

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Local Organising Committee

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Welcome Message

Welcome to the 70th International Congress of Meat Science and Technology (ICoMST), set to unfold in the picturesque setting of Foz do Iguassu, Brazil, from August 18th to 24th, 2024. We are thrilled to have you join us for this significant event, marking the second time ICoMST has graced the vibrant land of Brazil since its debut in Campinas in 2006.

The overarching theme for the 2024 congress is 'Responsible Meat Production,' a pivotal focus that will delve into various aspects of the meat chain from farm to fork. Encompassing discussions on production methodologies, sustainability, animal welfare, innovation, meat quality, safety, and consumer-related topics, the congress promises to be an enlightening exploration of the challenges and advancements within the realm of meat science and technology.

This event offers an outstanding opportunity for companies to engage in meaningful discussions with leading professionals in the field, enabling them to address challenges and share advancements that can contribute to enhancing the meat supply chain. Beyond the insightful scientific program, participating companies have the exclusive opportunity to spotlight their brands and products before influential professionals and industry leaders.

Furthermore, a social program is being crafted to provide an unforgettable experience coupled with picturesque landscapes, Brazilian culture, and cuisine. Companions will have the opportunity to explore the beauty of Foz do Iguaçu, including attractions such as the Bird Park, Itaipu Dam, and more.

Therefore, the congress not only provides a platform for professional development but also offers ample opportunities for networking and cultural exchange. We look forward to welcoming you to Foz do Iguassu, where scientific exploration seamlessly blends with the beauty of the surroundings, the richness of Brazilian cuisine, and the camaraderie of networking, ensuring a holistic and memorable experience at the 70th ICoMST.

Congress Chair
Saulo da Luz e Silva

Congress Co-Chair
Marco Antonio Trindade

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Keynote Speakers

FERNANDO SAMPAIO Brazilian Association of Meat Exporters - Brazil

SESSION 1: Responsible Meat Production

Agronomical Engineer, graduated from the Luiz de Queiroz College of Agriculture (1997), with a specialization in the meat and milk market from the École Supérieure d'Agriculture d'Angers in France. Sampaio has a rich international background, having worked in the meat market in France at Société des Viandes Bretagne Anjou – Soviba (2000 to 2001), and sat Meat Import Zandbergen Brothers BV (2001 to 2008). In 2009, he assumed the role of Sustainability Coordinator at the Brazilian Association of Meat Exporting Industries – ABIEC. His tenure extended from 2011 to 2016, during which he played a pivotal role in institutional representation, international negotiations, and commercial promotion, while concurrently advancing the sustainability agenda. Notably, Sampaio was instrumental in the formation and leadership of the Sustainable Livestock Working Group – GTPS from 2015 to 2016. He also contributed to the Global Roundtable for Sustainable Beef – GRSB and served on the Sustainability Committee of the International Meat Secretariat - IMS. In October 2016, Sampaio transitioned to the role of Executive Director of the Produce, Conserve, Include Strategy, a jurisdictional sustainability initiative in the State of Mato Grosso. His efforts in this role paved the way for a new phase with the establishment of the PCI Institute in early 2019. Starting from February 2023, Sampaio returned to ABIEC, taking on the position of Director of Sustainability. His extensive experience and commitment to sustainable practices continue to make a lasting impact in the industry.

ALEXANDRE BERNDT Brazilian Agricultural Researc Corporation (EMBRAPA) - Brazil

SESSION 2: Sustainability

Holds a degree in Biological Sciences from the University of São Paulo - USP (1993), a degree in Agronomic Engineering from ESALQ/USP (1998), a Master's degree in Animal Science and Pastures from ESALQ/USP (2001) and a PhD in Ecology of Agroecosystems from USP (2005). He exercised professional activity in Environmental Management in a multinational company. He was a scientific researcher at the Animal Science Institute of APTA-Agência Paulista de Tecnologia dos Agronegócios between 2007 and 2010. He is currently a researcher at EMBRAPA Sotheast Livestock in São Carlos, SP. He has experience in Animal Science, with emphasis on Sustainable Animal Production Systems, working mainly on the following topics: animal nutrition, nutritional requirements, meat quality and methane production in cattle. He participates in the Technical Committee of the Ministry of Science, Technology and Innovations - MCTI for the preparation of the National Inventory of Greenhouse Gas Emissions. He participates in the FAO Livestock Environmental Assessment and Performance Partnership-LEAP enteric methane working group. He participated in the Steering Committee of the LANDMARK Research Network of the European Union. He represents Embrapa in FAO's GASL-Global Agenda for Sustainable Livestock. Between 2016 and 2019 he held the chair of the international committee of the Conference on Greenhouse Gases and Animal Agriculture-GGAA, holding a meeting in Brazil in August 2019 (www.GGAA2019.org). He is a report reviewer for the IPCC and the World Bank. He served as Deputy Head of Research and Development at Embrapa Southeast Livestock between January 2014 and August 2021. He is currently Chief General of Embrapa Southeast Livestock and Vice President of the Brazilian Society of Animal Science.

Keynote Speakers

MARCIA DEL CAMPO GIGENA Instituto Nacional de Investigación Agropecuaria – Uruguay

SESSION 3: Animal Welfare

Marcia holds a degree in Agricultural Engineering from the Universidad de la República, Uruguay (1994). She earned her master's degree in Advanced Studies on Meat Quality from the Universitat Politècnica de València (2007) and completed her Ph.D. in Animal Welfare and Meat Quality from the same institution in 2009. Since 1994, she has served as the principal animal scientist at the National Institute of Agricultural Research. Currently, Marcia holds the position of President of the Directing Council of the National Institute of Animal Welfare (INBA) within the Ministry of Livestock and Agriculture (MGAP URUGUAY), a role she has held since its establishment in July 2021. Additionally, Marcia actively engages in livestock farming in the northern region of Uruguay.

Furthermore, Marcia has made significant contributions to education, having taught over 70 courses on Animal Welfare and Beef Quality in Uruguay and abroad over the last six years. She has also provided guidance to more than 50 students at both the undergraduate and graduate levels. Her scholarly output includes over 130 articles and contributions to national and international scientific events. Marcia has delivered more than 90 conferences in Uruguay and around the world. Since 2011, she has been responsible for the Ethical Commission for the Use of Experimental Animals at INIA Uruguay.

Marcia is a Guest Professor at the Public University in Uruguay, Faculty of Agronomy (since 2008) and a lecturer at São Paulo State University (since 2013). Additionally, she serves as the ICoMST contact person in Uruguay and is a member of various national and international working groups and scientific committees, including the World Organization for Animal Health (OMSA), the International Meat Secretariat (IMS), and the American Meat Science Association (AMSA).

ANDRZEJ SOSNICKI GenusPIC / USA

SESSION 4: Genetics & Physiology

Andrzej Sosnicki received his MS in Biology-Animal Physiology and Ph.D. in Agricultural Sciences-Muscle Biology & Meat Science, from University of Life Sciences in Poznan, Poland.

Andrzej's academic carrier includes appointments at: Department of Zootechnical Sciences, University of Life Sciences, Poznan, Poland; Muscle Biology & Meat Science laboratory, University of Wisconsin, Madison; Department of Zoology, University of Tennessee, Knoxville, and Department of Biology, University of Pennsylvania, Philadelphia. His academic research focused on skeletal muscle morphology, pathology and meat quality, and he studied physiological principles of skeletal muscle function during locomotion. Andrzej also held Adjunct Associate Professor positions at Department of Animal Science, Iowa State University, Ames, IA in 2002-2010, and at Department of Animal & Avian Sciences, University of Maryland, College Park, MD in 2016-2019.

Andrzej's industry experience begun in 1990 at the R&D Department of Oscar Mayer Foods Corporation/ Kraft Foods, Madison, WI, where he managed research focusing on creating value in vertically integrated poultry & pork production and meat processing systems. In 1995 he joined PIC to establish and manage the company's Muscle Biology & Applied Meat Science endeavors emphasizing the commercial importance of pig carcass and meat quality traits in the PIC's genetic improvement program. Over the years he has held several technical and P&L/commercial positions in North and Latin America and in the EU. He is currently Director of Global Technical Projects focusing on further integration of pig skeletal muscle growth-physiology and meat quality into the matrix of GenusPIC's genetic improvement program.

Dr. Sosnicki is the author/co-author of over forty publications, six book chapters and several scientific abstracts. He received the American Meat Institute Distinguished Extension-Industry Service Award in 2013 and the American Meat Science Association International Lectureship Award in 2015.

Keynote Speakers

TRACY SCHEFFLER University of Florida / USA

SESSION 5: Muscle biology & Meat quality

Tracy's academic carrier includes appointments at Virginia Tech as Lab Specialist and as Assistant Professor in the Department of Animal Sciences, at University of Florida, where now she is Associate Professor in muscle biology. She is the lead instructor for Experimental Techniques in Meat Science, which exposes students to laboratory methods in meat science and muscle biology. She also teaches part of Meat Technology course, that covers topics in meat science from muscle structure, growth, and meat quality development to microbiology and food safety. As a researcher, the overall goal of Dr. Scheffler's research is to optimize muscle growth, composition, and meat quality. In particular, she is interested in how muscle phenotype, metabolic properties, and energy signaling pathways influence the following; protein turnover and growth of skeletal muscle, and impact on production parameters, such as lean gain, nutrient partitioning and muscle and whole body composition, and the conversion of muscle to meat and development of meat quality attributes. During her career, Tracy won great number of awards: Outstanding Dissertation Honorable Mention in Science and Engineering, from Virginia Tech in 2013, E.T. Kornegay Outstanding Ph.D. Student Award from the Department of Animal and Poultry Sciences, Virginia Tech in 2011, Featherston Early Graduate Career Award from the Department of Animal Sciences, at Purdue University in 2008 and also a Featherston Graduate Student Award for Outstanding Teaching, Department of Animal Sciences from Purdue University in 2005. Her scientific contributions are abundant as she author/co-author over forty scientific papers, including recent reviews related to Bos indicus beef quality. Among her publications, there are also book chapters as well as lab protocol. Most recently, in 2021 Tracy won an Achievement Award by the AMSA due to her dedication to cutting-edge research on improvement of the quality and palatability of beef from Bos indicus influenced cattle.

MINDY BRASHEARS Texas Tech University / USA

SESSION 6: Meat safety

Mindy Brashears is the former Under Secretary for Food Safety at the U.S. Department of Agriculture. She was nominated by President Donald J. Trump and confirmed by a Senate vote on March 23, 2020 and concluded her service on January 20, 2021. Her responsibilities in this role included leading the nation's Food Safety and Inspection Service (FSIS) and its team of over 10,000 food inspectors and scientists. She chaired the Codex Alimentarius Policy Committee, which made her the highest-ranking food safety official in the U.S. government during her tenure. Following her time at USDA, she returned to her role as Professor of Food Microbiology and Food Safety at Texas Tech University where she is the director for the International Center for Food Industry Excellence.

GRAHAM E. GARDNER Murdoch University – Australia

SESSION 7: Objective measurement of carcass and meat quality

Graham's research focuses on physiological responses to selection for growth, leanness and eating quality within the sheep, pork, and cattle industries, and the development of technologies that measure these traits. Graham is the Chief Investigator of the Advanced Livestock Measurement Technologies Project (ALMTech) which is a national collaboration focused on the development & commercialisation of objective carcase measurement technologies. Within Australia this project has driven the development and roll-out of a range of different technologies that measure lean meat yield and eating quality in livestock.

Keynote Speakers

MUSTAFA FAROUK AgResearch – New Zealand

SESSION 9: Meat products development

Dr Mustafa Farouk is a Senior Meat/Food Scientist in the Food Technology and Processing Group of AgResearch Ltd, Hamilton, New Zealand. His research activities help build platforms for technologies and industry engagements to support the creation and export of high-value meat-based ingredients for manufacturers, and protein-rich functional foods for consumers having optimised nutritional composition, organoleptics, and physiological benefits. He graduated with a Masters and PhD in Food Science from Michigan State University, USA. His Research interest is in exploiting the functional properties of muscle proteins for gelation and texturization purposes; non-traditional uses of meat proteins, halal meat and food production, quality of manufacturing beef; adding value to red meats and meat products; and improving the understanding of meat as a central ingredient in foods. Farouk is a member of many professional bodies and sits on the advisory committees of governmental and non-governmental organizations related to foods, and has published extensively on meat and meat products.

LUCA SIMONE COCOLIN University of Torino/ Italy

SESSION 10: Meat products stability

Luca Simone Cocolin is a distinguished academician holding the position of Full Professor in the Department of Agricultural, Forest and Food Sciences at the University of Torino, Italy. He has authored over 300 publications in peer reviwed journals. His expertise spans diverse domains, encompassing the development, optimization, and application of molecular methods for detecting, quantifying, and characterizing foodborne pathogens. Additionally, his research delves into the microbial ecology of fermented foods, bioprotection, and the study of the human microbiome.

Institutionally, Professor Cocolin has assumed significant roles, including his current position as the President of the Degree Course on Food Science and Technology since 2015 at the University of Torino. He has been a stalwart member of the Management Board of the PhD in Agricultural, Forest and Food Sciences since 2010 and has actively contributed to the Orientation, Tutoring, and Job Placement Committee of the Department of Agriculture, Forest and Food Sciences since 2008.

Since 2001, Professor Cocolin has played pivotal roles in numerous national and European projects, serving as a project or research unit coordinator. Furthermore, his editorial contributions include being the Editor in Chief of the International Journal of Food Microbiology since 2008, Academic Editor of PLOS One since 2016, and a member of various editorial boards, attesting to his commitment to advancing scientific knowledge.

Demonstrating his global engagement, Professor Cocolin has been an Executive Board Member of the International Committee on Food Microbiology and Hygiene (ICFMH) since 2008, contributing to the International Union of Microbiological Societies (IUMS). His involvement extends to leadership roles in the European Technology Platform Food for Life, the Scientific Advisory Board of Arla Foods Dairy Health and Nutrition Excellence Center, and the EIT Food initiative.

Moreover, he has lent his expertise to numerous international research project evaluations for esteemed organizations such as the French National Research Agency (ANR), the Research Foundation Flanders, the Istitut National de la Recherce Agronomique (INRA), the Skolkovo Foundation, the Vienna Science and Technology Fund (WWTF), and various Italian ministries and agencies.

Keynote Speakers

STEFAAN DE SMET Ghent University - Belgium

SESSION 11: Meat and Health

Stefaan De Smet graduated in 1986 as an Agricultural Engineer from Ghent University, specializing in Animal Production. Since 1987, he has been associated with the Department of Animal Production at Ghent University. Initially, he joined as a PhD student and later served as a research project assistant and post-doctoral scientist before assuming the role of an assistant professor. His research interests are related to the domain of meat quality, with a keen focus on understanding the correlation between carcass quality and sensory and technological traits of meat quality. Presently, he holds the position of a full professor in Animal Science at Ghent University, Belgium. In this capacity, he is responsible for teaching both general and advanced courses in Animal Production, Animal Breeding, and Meat Science. His ongoing research is dedicated to exploring meat quality and the health value of meat and meat products, with a specific emphasis on optimizing the composition and oxidative stability of meat from different species. Stefaan De Smet also serves as the current president of the Belgian Association of Meat Science and Technology (BAMST). As an accomplished academic, he is (co-)author of over 150 scientific publications in peer-reviewed journals. Additionally, he actively contributes to the academic community by regularly refereeing manuscripts for several journals.

MARIA FONT I FURNOLS IRTA - Spain

SESSION 13: Consumer topics

Maria Font i Furnols is MSc Agriculture Engineer (1995), MSc in Food Science and Technology (1997) and MSc in Psychology (2011). She obtained her PhD in 2000 in the Department of Statistics and Operative Investigation of the Polytechnic University of Catalonia working in an European project about boar taint mainly in trained panel and consumer aspects. She works as researcher at IRTA since 2000 and nowadays belongs to the program of Food Quality and Technology. She works in carcass grading, carcass quality and meat quality considering aspects related to the productive system and the characteristics of the animals and in consumer studies. She has used the computed tomography to evaluate the body composition of life animals, carcasses and cuts. She has participated and lead several national and international projects. She has more than 100 scientific publications and more than 50 technical papers, as well as several book chapters.

Keynote Speakers

DECLAN TROY TEAGASC/ Ireland

SESSION 14: Meat science communication

Declan completed his post graduate studies at University College Dublin in 1986 investigating the biochemistry of muscle proteases as a function of pH. He later became Head of the Meat Science Department in The National Food Centre, Dublin. During this time he has published over 150 scientific peer reviewed publications, book chapters and scientific articles, mainly in the area of meat quality. The main focus of his research is on the biochemistry of muscle proteins and their role in meat tenderness. Declan has always encouraged the up-take of science based innovations by the food industry and has interacted widely with the sector to this end. He has collaborated in his research programme with many different research groups from Europe and all around the world including Australia, Korea, New Zealand, Uruguay, China, Brazil and of course the USA.

Declan has fostered highly successful international collaborations and exchange of knowledge in food science by leading research projects worth more than €80 million that supported 125 PhD students globally in different laboratories at collaborating institutions funded nationally and internationally. Most recently he has been appointed as the Director of the Consumer Food Centre in Teagasc.

Declan sits on many national and international committees formulating research priorities in food science and advising state agencies and companies. He is currently a member of the UNECE Working Group on Meat Quality. He was Chairman of the World Congress of Food Science and Technology 2016 (IUFoST 2016) in Dublin in his capacity as President of the Institute of Food Science and Technology of Ireland. He has also been appointed the International Secretary of the annual International Congress of Meat Science and Technology (ICoMST) of which he was chair both in 2006 (Dublin) and Cork (2017) and he is Academic Lead to Meat Technology Ireland (MTI). Currently he is manages the Teagasc Research Centre in Ashtown, Dublin.

Full Program

Sunday

18 August

15:00 – 20:00 | Registration desk opens

18:00 – 21:00 | Welcome reception

Monday

19 August

09:00 – 09:30 | Opening ceremony

09:30 – 10:20 | SESSION 1: Responsible Meat Production

Cultivating responsibility: Brazil's approach to sustainable meat production

Fernando Sampaio (ABIEC, Brazilian Association of Meat Exporters – Brazil)

Chairs: Alessandra Fernandes Rosa and Amilton de Mello

10:20 – 11:20 | Coffee-Break & Poster viewing

11:20 – 12:10 | SESSION 2: Sustainability

Low carbon systems and their impacts on meat yield and quality

Alexandre Berndt (EMBRAPA, Brazilian Agricultural Research Corporation/Brazil)

Chairs: Angélica Simone Cravo Pereira and Phillip Strydom

12:10 – 12:40 | Short Paper Oral presentations

Exploring sustainable feeding solutions for the native \'porco celta\' breed with local resources inclusion

Noemí Echegaray – Centro Tecnolóxico da Carne, Spain

Artichoke bracts silage in beef cattle diet: meat quality during dry aging

Aristide Maggiolino – University of Bari, Italy

12:40 – 14:30 | Lunch & Poster viewing

14:30 – 15:20 | SESSION 3 Animal Welfare

Future livestock production: animal welfare as a key component of sustainability and beef quality

Marcia del Campo (INIA, Instituto Nacional de Investigación Agropecuaria - Uruguay)

Chairs: Antonella Dalle Zotte and Fabio Montossi

15:20 – 15:50 | Short Paper Oral presentations

The effect of different cattle marketing alternatives on the eating quality of beef

Peter McGilchrist – University of New England, Australia

R-zeta® additive fattening diets impact on meat quality cattle

María Eugenia Munilla – National Agricultural Technology Institute (INTA), Argentina

15:50 – 16:50 | Coffee-Break & Poster viewing

16:50 – 17:40 | Session 4: Genetics and Physiology

A review of porcine skeletal muscle plasticity and implications for genetic improvement of carcass and meat quality

Andrzej Sosnicki (GenusPIC/USA)

Chairs: Robin Warner and Cristiano Sales Prado

17:40 – 18:10 | Short Paper Oral presentations

Early post-mortem discrimination between beef tenderness classes in feedlot nellore bulls using muscle lipid biomarkers

Daniel Antonelo – Lipid Marker Omics Sciences, Brazil

Impact of visual dark-cutting severity and aging on the metabolomic profile of beef longissimus lumborum

Keayla Harr – Oklahoma State University,USA

19:00 – 22:00 | Typical dinner

Tuesday

20 August

09:00 – 09:50 | SESSION 5: Muscle biology & Meat quality

Post mortem proteolysis and metabolism in Bos indicus beef

Tracy Schefler (University of Florida/USA)

Chairs: David Gerrard and Peter Purslow

Full Program

20 August

09:50 – 10:20 **Short Paper Oral presentations**

Dry-aging impacts on color, fatty acid, and lipid oxidation of striploin from dairy crossbred yearling and 2-year-old cattle

Renyu Zhang – AgResearch Ltd, New Zealand

Proteolysis in vitro reveals calpain-1 activity during the beef maturation process

Jocelyn Bodmer – Virginia Tech, USA

10:20 – 11:20 **Coffee-Break & Poster viewing**

11:20 – 12:10 **SESSION 6: Meat Safety**

Salmonella issues related to meat safety

Mindy Brashears (Texas Tech University/USA)

Chairs: Maria João Fraqueza and Theo Verkleij

12:10 – 12:40 **Short Paper Oral presentations**

Effect of lactic acid, UV-C radiation and vacuum packaging on Listeria monocytogenes, Salmonella spp., Pseudomonads spp. and Lactic acid bacteria growth on raw chicken breasts.

Caterina Rufo – Universidad de la Republica, Uruguay

Spray-on application of food cultures for safety and quality improvement of cooked, cured meat products

Jens K.S. Møller – Novonesis, Denmark

12:40 – 14:30 **Lunch & Poster viewing**

13:45 – 14:30 **Elsevier Symposium – Meat Science Journal Author Workshop**

14:30 – 15:20 **SESSION 7: Objective measurement of carcass and meat quality**

Technologies for determining lean meat yield and eating quality and their industrial accreditation

Graham Edwin Gardner (Murdoch University/Australia)

Chairs: Igor Tomasevic and Mario Chizzotti

15:20 – 15:50 **Short Paper Oral presentations**

Beef fecal detection using a fluorescence multispectral camera system and deep learning object detection algorithml

Juntae Kim – Chungnam National University, South Korea

The use of dual-energy x-ray absorptiometry (dxa) for predicting total and intramuscular fat in pork loin steaks

Jenifer Ramos – Universidade Federal de Viçosa, Brazil

15:50 – 16:50 **Coffee-Break & Poster viewing**

16:50 – 17:50 **SESSION 8: Bridging Industry and Science for Future Innovations – From farm to slaughter**

Chair: Prof David Hopkins

Sustainable Solutions for the Meat Industry: Facing Challenges and Embracing Opportunities

Liège Vergili Correia Nogueira (JBS)

Innovating the Future: Recent Breakthroughs and Challenges in Meat Industry Automation

Frans van der Steen (Marel)

19:00 – 22:00 **BBQ dinner**

21 August

Wednesday

Technical and scenic tours

22 August

Thursday

09:00 – 09:50 **SESSION 9: Meat Products Development**

Tailoring Meat Products for the Elderly: A Comprehensive Review

Mustafa Farouk (AgResearch/ NZ)

Chairs: Ana Lúcia da Silva Corrêa Lemos and Pere Gou

Full Program

22 August

09:50 – 10:20 **Short Paper Oral presentations**

Fermentation of pea and soy protein with Lactilactobacillus plantarum improves flavor of plant-based burger patties

Jens K.S. Møller – Novonesis, Denmark

Effect of smoking on the texture of different casing-types of frankfurters

Malte Leible – University of Hohenheim, Germany

10:20 – 11:20 **Coffee-Break & Poster viewing**

11:20 – 12:10 **SESSION 10: Meat Products Stability**

Bioprotective cultures in meat products

Luca Simone Cocolin – (University of Torino/ Italy)

Chairs: Renata Emlund Freitas de Macedo and Tommy Wheeler

12:10 – 12:40 **Short Paper Oral presentations**

Influence of air contact and pre-packaging treatment on the color stability of vacuum packaged beef

Johannes Krell – University of Hohenheim, Germany

Addition of freeze-dried beef exudate alters volatile flavor profile of cooked ground beef patties

Jerrad Legako - Texas Tech University, USA.

12:40 – 14:30 **Lunch & Poster viewing**

14:30 – 15:20 **SESSION 11: Meat and Health**

Meat products as “hazard”/”risk”

Stefaan De Smet – (Ghent University /Belgium)

Chairs: Claudia Ruiz-Capillas and Sghaier Chriki

15:20 – 15:50 **Short Paper Oral presentations**

Salt reduction strategies for meat products

Patrícia Bernardo – University of Lisbon, Portugal

Adding an antioxydant cocktail to pig feed reduced luminal oxidation in rats fed a cooked ham diet from supplemented rearing

Aurélie Promeyrat – Institut du Porc, France

15:50 – 16:50 **Coffee-Break & Poster viewing**

16:50 – 17:50 **SESSION 12:**

Chair: Dr. Lars Leopold Hinrichsen

Transforming Demands into Research: Integrating Science in the Meat Industry

Carlos Alberto Guerra - BRC

New Meat Tech: why & how state-of-the-art research matters in the meat industry

Victor M. S. Franco – BRF

19:30 – 00:30 **Gala dinner**

Friday

23 August

09:00 – 09:50 **SESSION 13: Consumer Topics**

An overview of drivers and emotions of meat consumption

Maria Font i Furnols (IRTA/Spain)

Chairs: Rhonda Miller and Tania Ngapo

09:50 – 10:20 **Short Paper Oral presentations**

Sensory traits importance driven by the beef consumers: pathways to a 3g global beef eating quality predictive system to meet consumers’ expectations

Alix Neveu – International Meat Research 3G Foundation (IMR3GF), Poland

Microbiological and sensory acceptability of ham: effect of high pressure and biopreservation

Laurence Pottier – ONIRIS, France

10:20 – 11:00 **Coffee-Break & Poster viewing**

11:00 – 12:00 **SESSION 14: Meat science communication**

Round table: Sustaining the future of meat science

Chair: Declan Troy - (TEAGASC/ Ireland)

Speakers: Collette Kaster, Frédéric Leroy, Keith Belk, Mark Lyons and Sérgio Pflanzer

12:00 – 12:30 **Closing ceremony**

Social Programme

18 August

Sunday
Welcome reception

19 August

MONDAY
Typical dinner

20 August

Tuesday
BBQ dinner

21 August

Wednesday
Technical and scenic tours

22 August

Thursday
Gala dinner

23 August

Friday
Closing ceremony

Technical & Scenic Tours

All registered ICoMST 2024 participants are entitled the opportunity to select one of the provided tours which are described below. The following Technical and Scenic Tours are included in the Congress registration fees, for all registered Delegates,Students/Young Researchers and Accompanying persons.

Tour occupancy is subject to availability, on a first come, first served basis.
The tours were organized to provide all participants with the opportunity to visit the breathtaking Iguassu Falls.
Please note this list could suffer possible changes and/or updates!

IMPORTANT!
All tours will depart from the Bourbon Cataratas Hotel at the scheduled time. Don't forget to bring your badge.
More information on the schedule of each tour will be available at ICoMST 2024 webpage later.

Tour 1

Panoramic Itaipu dam (external area) & Iguassu falls

Explore the external surroundings of Itaipu Dam in the morning, encompassing the entire external area of the facility, including the Avenue of Forced Conduits (White Tubes) and Dam Top. Following this, enjoy lunch at Cataratas Falls restaurant and experience the awe-inspiring Iguassu Falls with a round-trip inside the park (12 km), a stroll along the Cataratas Trail (1 km - optional), and visits to the elevator, walkway, and various viewpoints. *Wednesday, 21 August 2024*

Tour 2

Internal and external Itaipu dam (internal and external areas) & Iguassu falls

In the morning, seize the opportunity to explore the core of the Itaipu Dam. Delve into not only the external surroundings but also the interior of the dam, journeying to the engine room. Here, you can marvel at the Forced Conduits, the Concrete Cathedrals, and the Turbine Axis. At the conclusion of this tour you will savor lunch at Cataratas Falls restaurant and immerse the awe-inspiring Iguassu Falls with a round-trip inside the park (12 km), a stroll along the Cataratas Trail (1 km - optional), and visits to the elevator, walkway, and various viewpoints. *Wednesday, 21 August 2024*

Technical & Scenic Tours

Tour 3
Iguassu Falls & Itaipu dam


Start your morning exploring the wonders of the Iguaçu Falls with a complete journey inside the park (12 km), a walk along the Falls Trail (1 km - optional), and visits to the elevator, walkway, and various viewpoints. After that, enjoy a delicious lunch at the Iguassu Restaurant, within the park. In the afternoon, explore the core of the Itaipu Dam. Delve not only into the external surroundings but also into the interior of the dam, heading to the engine room. Here, you can marvel at the Forced Conduits, the Concrete Cathedrals, and the Turbine Axis. *Wednesday, 21 August 2024*

Tour 4
Bird Park & Iguassu falls


Embark on a morning visit to the Bird Park, where you'll step into expansive aviaries to observe toucans, parrots, jacutingas, and other birds up close. Immerse yourself in the vast biodiversity of the Atlantic Forest. In the afternoon, explore the awe-inspiring Iguassu Falls, featuring a round-trip journey inside the park (12 km), a walk along the Cataratas Trail (1 km), and visits to the elevator, walkway, and various viewpoints. Wednesday, 21 August 2024. *Wednesday, 21 August 2024*

Tour 5:
Pig slaughterhouse/meat processing plant & Iguassu falls


In the morning, embark on a tour of a pig processing plant (https://www.friella.com.br), gaining insights into the pig slaughter and meat processing facilities while becoming acquainted with and savoring typical Brazilian products. After the tour, indulge in lunch at Cataratas Falls restaurant. In the afternoon, submerge yourself in the awe-inspiring Iguassu Falls with a round-trip journey inside the park (12 km), an optional 1 km stroll along the Cataratas Trail, and visits to the elevator, walkway, and various viewpoints.

Technical & Scenic Tours

Tour 6:
Meat products research lab & Iguassu falls

In the morning, explore the Federal Technological University of Paraná (Medianeira campus), which includes a visit to the meat products pilot plant and a tasting of typical Brazilian meat products. Following the tour, enjoy lunch at Cataratas Falls restaurant. In the afternoon, immerse yourself in the awe-inspiring Iguassu Falls with a round-trip journey inside the park (12 km), an optional 1 km stroll along the Cataratas Trail, and visits to the elevator, walkway, and various viewpoints.

Tour 7
Pork processing company & Iguassu falls

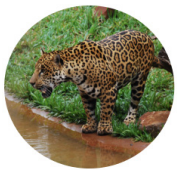
In the morning, embark on a visit to a pig processing company (https://www.frimesa.com.br/en/) with the unique experience of a virtual tour of its internal facilities using 3D glasses, followed by a tasting of meat products. Following the tour, treat yourself to lunch at Iguassu Falls restaurant. In the afternoon, explore the stunning Iguassu Falls, featuring a round-trip journey inside the park (12 km), a walk along the Trail (1 km - optional), and visits to the elevator, walkway, and various viewpoints.

Accompanying Person Programme

- Welcome Reception (Sunday, August 18)
- BBQ Dinner (Monday, August 19)
- Typical Dinner (Tuesday, August 20)
- Technical & Scenic Tours (Wednesday, August 21)
- Gala dinner (Thursday, August 22)
- Additional tourist activities as follows:
- Please note this list could suffer possible changes and/or updates!

IMPORTANT!
All tours will depart from the Bourbon Cataratas Hotel at the scheduled time. Don't forget to bring your badge.
More information on the schedule of each tour will be available at ICoMST 2024 webpage later.

Bela Vista Biological Refuge




The Bela Vista Biological Refuge encompasses an area of 1,780 hectares dedicated to the preservation of native species of fauna and flora. Currently, the refuge is integrated and recognized as an outpost of the Atlantic Forest Biosphere Reserve and forms part of the Paraná River Biodiversity Corridor, connecting the Iguaçu National Park to the protected areas of Itaipu and Ilha Grande National Park. The Roberto Ribas Lange Zoo, the main attraction of the refuge visitation itinerary, houses 189 animals from 52 species, including reptiles, amphibians, birds, and mammals. The animals are sourced from Itaipu's own wildlife breeding center or other environmental protection agencies. *Monday, 19 August 2024*

Bird Park & Marco of the 3 Frontiers


The Bird Park, South America's largest, is dedicated to conserving Atlantic Forest birds, housing over 150 species. Visitors can explore four immersive aviaries, getting close to toucans, hummingbirds, and macaws. The park also features a wetlands area with reptiles like crocodiles and a boa constrictor. As visitors stroll, they encounter diverse bird species and learn about conservation efforts. In the same day trip, tourists can visit the Marco of the 3 Frontiers, where the obelisk stands as a symbol of peace and cooperation among Brazil, Argentina, and Paraguay. Erected in 1903, it marks the sovereignty and territorial boundaries. This site offers a panoramic view of the borders, showcasing rich culture and unique geography. *Tuesday, 20 August 2024*

Accompanying Person
Programme

City tour & Shopping

Explore the external surroundings of Itaipu Dam in the morning, encompassing the entire external area of the facility, including the Avenue of Forced Conduits (White Tubes) and Dam Top. Following this, enjoy lunch at Cataratas Falls restaurant and experience the awe-inspiring Iguassu Falls with a round-trip inside the park (12 km), a stroll along the Cataratas Trail (1 km - optional), and visits to the elevator, walkway, and various viewpoints. *Thursday, 22 August 2024*

Abstracts of short Oral Communications

SESSION 2

Sustainability

Monday 19 August 2024

EXPLORING SUSTAINABLE FEEDING SOLUTIONS FOR THE NATIVE 'PORCO CELTA' BREED WITH LOCAL RESOURCES INCLUSION

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I. INTRODUCTION

The increasing competitiveness in the meat market has generated a growing need to produce high-quality products. In this context, the recovery of native breeds like 'Porco Celta' plays a crucial role. Despite its slower growth, this breed provides excellent meat quality while allowing for the preservation of genetic heritage and biodiversity, making it a valuable option for discerning consumers [1]. Additionally, the use of native breeds is often associated with extensive production systems and traditional feeding practices that respect the environment due to its lower environmental footprint and reduced impact on local ecosystems [2]. Therefore, our work focuses on developing feed for slow-growing pig breeds such as 'Porco Celta', utilizing local raw materials. This approach aims to reduce dependence on ingredients such as soy, whose cultivation is linked to deforestation, soil degradation, wildlife habitat destruction, and loss of natural grasslands [3], while ensuring that meat quality is not compromised using locally sourced ingredients that are more environmentally friendly.

II. MATERIALS AND METHODS

To conduct this research, 20 'Porco Celta' pigs were used after a prior 3-month feeding period with a starter feed. The animals were randomly divided into two groups: a control group of 10 pigs (5 males and 5 females) fed a commercial diet consisting of barley, soy, wheat, and corn; and a group of 10 pigs (4 males and 6 females) fed a formulation composed of corn (27%), wheat (21%), peas (20%), soy (11%), starch bran (10%), rapeseed (6%), and flaxseed (1%). The fattening period lasted for 7 months under extensive regimen. All analyses were conducted on the *Longissimus dorsi* (LD) muscle. Moisture, protein (Kjeldahl N × 6.25), and ash were determined following ISO standards, while fat content was assessed using the American Oil Chemists' Society (AOCS) procedure. Meat quality analysis, including pH and color measurement (24 h post-slaughter), water holding capacity (WHC), and texture analysis (Warner-Bratzler test), was performed following the methodology described by Pateiro et al. [4]. To assess the effects of feeding regimen and the potential interaction between feeding and sex, an analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure in SPSS version 23.0.

III. RESULTS AND DISCUSSION

As indicated in Table 1, the diet provided to 'Porco Celta' had no significant effect ($P > 0.05$) on the chemical composition of the LD muscle, except for the moisture content in females, which was significantly higher ($P < 0.01$) in pigs fed the reformulated diet with increased incorporation of local raw materials.

Table 1 – Effect of a reformulated diet on the chemical composition of 'Porco Celta' *Longissimus dorsi* muscle (values expressed as mean ± standard error)

	Males			Females			
	Control diet	Reformulated diet	Sig.	Control diet	Reformulated diet	Sig.	DxS
Moisture (%)	72.10 ± 1.12	75.59 ± 0.35	ns	69.73 ± 1.23	71.74 ± 1.40	**	ns
Intramuscular fat (%)	4.01 ± 1.52	3.27 ± 0.46	ns	6.97 ± 1.83	4.12 ± 2.21	ns	ns
Protein (%)	22.72 ± 0.52	23.29 ± 0.26	ns	22.11 ± 0.91	22.97 ± 1.11	ns	ns
Ash (%)	1.14 ± 0.03	1.16 ± 0.02	ns	1.15 ± 0.06	1.19 ± 0.07	ns	ns

Sig: significance: ** ($P < 0.01$); ns: no significant difference; DxS: interaction of diet and sex.

This finding is significant, considering that monogastric animals like pigs often experience alterations in muscle composition, particularly in fat content, when their diet is modified [5]. However, in this trial, despite modifications to the raw materials used in the reformulated feed, its proximal composition closely resembled that of the commercial feed, including similar concentrations of essential amino acids. Consequently, animal metabolism can remain largely unaffected. As observed in Table 2, the pH was significantly (P < 0.01) affected by the diet only in females, where the reformulated diet increased its value. Similarly, the L* parameter was significantly (P < 0.01) affected by the diet only in females. Specifically, the LD muscle from the reformulated diet showed the lowest L* values, suggesting less luminosity in the meat. As for the a* parameter, the reformulated diet in this case significantly (P < 0.01) affected males, who exhibited lower a* values, implying less redness in the meat. Both sexes exhibited a consistent pattern with the reformulated diet regarding the b* value, demonstrating a notable decrease in this parameter. Contrary, WHC and shear force were not affected by the diet, suggesting that both diets provide meat with similar juiciness and texture.

Table 2 – Effect of a reformulated diet on the physicochemical parameters of 'Porco Celta' *Longissimus dorsi* muscle (values expressed as mean ± standard error)

	Males			Females			
	Control diet	Reformulated diet	Sig.	Control diet	Reformulated diet	Sig.	DxS
pH	5.58 ± 0.13	5.67 ± 0.11	ns	5.42 ± 0.08	5.66 ± 0.11	**	ns
Color parameters							
L*	48.64 ± 0.45	48.19 ± 1.91	ns	54.22 ± 5.42	47.79 ± 3.07	**	ns
a*	5.21 ± 1.06	2.60 ± 0.60	**	3.00 ± 1.28	2.80 ± 10.21	ns	ns
b*	12.17 ± 0.68	9.87 ± 0.32	**	11.87 ± 0.91	10.21 ± 1.29	**	ns
Water holding capacity (%)	23.42 ± 4.09	24.52 ± 2.65	ns	25.12 ± 3.84	22.24 ± 3.46	ns	ns
Shear force (N/ cm^2)	47.23 ± 14.14	47.27 ± 9.01	ns	35.87 ± 10.00	51.46 ± 9.57	ns	ns

Sig: significance: ** (P < 0.01); ns: no significant difference; DxS: interaction of diet and sex.

IV. CONCLUSION

The reformulated diet with higher inclusion of local raw materials had minimal impact on the chemical composition of LD muscle of 'Porco Celta', as well as in its WHC and texture. However, the color parameters of the meat were affected overall, highlighting the need for future sensory evaluations in this aspect to ensure proper consumer acceptance.

ACKNOWLEDGEMENTS

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ARTICHOKE BRACTS SILAGE IN BEEF CATTLE DIET: MEAT QUALITY DURING DRY AGING

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I. INTRODUCTION

Valuing the agro-industrial byproducts most generated within a given territory holds significance for both economic and environmental reasons [1]. Depending on plant species and tissue types, horticultural and fruit waste exhibit a wide array of properties [2]. Artichoke bracts, for instance, constitute a waste material that can be repurposed for animal feed, thereby transforming them from waste into a resource and reducing food-feed competition. Adding artichoke bracts in beef cattle diet could increase the environmental sustainability and enhance the quality and the stability of meat, although the effects could differ during an aging process. The present study was conducted to assess the quality of beef from cattle fed with artichoke bract silage (BS) during a period of dry aging.

II. MATERIALS AND METHODS

Approval number ethic committee 2256-III/13. For the trial, 48 Holstein×Blue Belgian heifers were randomly assigned to 3 experimental groups: a control group (C) and 2 experimental groups receiving two different percentages of forage substitution with BS (S1: 6% on dry matter; S2: 12% on dry matter) for 60 days before slaughter. C, S1 and S2 diets were isoenergetic and isoproteic. After slaughter, the left loin was removed from each animal and subjected to dry aging for 42 days. At each experimental time (0d, 14d, 21d, 28d, 35d, 42d) a 30mm rib was cut from each loin for the analysis. Color, cooking loss (CL) and water-holding capacity (WHC) were estimated as described by De Palo et al. [3]. Thiobarbituric acid reactive substances (TBARS) and protein carbonyls determinations were performed as reported by De Palo et al. [4]. The ferric reducing antioxidant power (FRAP) assay was used to measure total antioxidant potential according to the method described by Dinardo et al. [5]. Textural properties were assessed using a ZI.0 TN texture analyzer (ZwickRoell GmbH & Co. KG, Ulm, Germany), equipped with 1 kN load-cell and a compression probe of 36 mm diameter, following the method outlined by De Angelis et al. [6] with few modifications. Samples were cooked in a plastic bag in a water bath at 85°C until an internal temperature of 75°C was reached. Shear force was measured on parallelepiped-shaped samples of 3 × 6 × 6 cm, sectioned so that the longitudinal axes was parallel to the orientation of muscle fibers. Each sample was cut three times, and the average of these three values was calculated. The results were expressed in N. Hardness, cohesiveness, springiness and chewiness [6] were evaluated on cubic meat samples of 1 cm per side. For these parameters a double compression cycle was performed at 1 mm s−1 until a recorded deformation of 75%. Three replicates for each sample were considered. The data set was tested for normal distribution and variance homogeneity (Shapiro-Wilk). Then, was subjected to analysis of variance (ANOVA), using the General Linear Model (GLM) software by SAS (2018) (version 9.3, SAS Institute Inc., Cary, NC, USA), according to the following model: $y_{ijk} = \mu + \alpha_i + G_j + T_k + (G \times T)_{jk} + \varepsilon_{ijkl}$, where y_{ijk} represents all parameters as dependent variables; μ is the mean; α_i is the single block random effect, G represents the effect of the j^{th} group, T represents the effect of the k^{th} aging time (k = 1, ..., 6), G × T represents the effect of the binary interaction between the two independent variables (jk = 1,…, 18) and ε_{ijkl} is the error. Subsequently, a Tukey test for repeated measures was carried out to evaluate the differences between the means during the aging time. All means were expressed as square means and mean standard error. The significance level was set to $P < 0.05$.

III. RESULTS AND DISCUSSION

Diet plays a primary role in the physicochemical regulation and metabolic traits of muscular development in livestock animals, thereby contributing significantly to the nutritional quality, organoleptic characteristics, and shelf life of meat [7]. In our results, lightness values of C meat increased from 0d to 21d (35.59 to 38.54), declining thereafter at 42d (35.15) ($P < 0.01$). Moreover, at 42d, these values were consistently lower compared to those of S1 and S2 meat (35.15 *vs.* 37.65 and 37.70) ($P < 0.01$). Lightness is closely associated with the chemical composition of meat, particularly to intramuscular fat concentration and composition, as well as water content [8]. The WHC of S1 meat increased from 0d to 21d (73.98 to 83.28) ($P <0.01$), whereas in S2 meat it increased from 0d to 14d (75.66 to 79.96) ($P <0.01$). Subsequently, WHC values for both meat S1 and S2 remained stable ($P < 0.01$). Although at 21d the WHC of C meat was lower than that of S1 meat (78.28 *vs.* 83.28) ($P < 0.01$), the CL showed lower values in C meat compared to S2 at 28d (31.85 *vs.* 35.66) and to both S1 and S2 at 42d (25.68 *vs.* 29.96 and 30.23) ($P < 0.01$). Regarding oxidative patterns, TBARS revealed statistically significant differences over time only in C meat, increasing from 0d to 14d (0.17 to 0.34) and remaining constant thereafter ($P <0.01$). Protein carbonyls increased from 0d to 35d in S2 meat (1.44 to 2.13) ($P < 0.01$), while FRAP values showed no statistical difference between time points or groups ($P > 0.05$). Shear force values showed the same trend in meat of all three groups, decreasing from 0d to 14d and remaining constant thereafter (from 57.89-48.88 to 25.88-18.96) ($P < 0.01$). None of the texture parameters revealed any differences between aging times or groups ($P > 0.05$).

IV. CONCLUSION

Based on these results, it can be stated that the use of BS in heifers' diet did not compromise the qualitative characteristics of the dry-aged loins. On the contrary, it could enhance WHC and oxidative stability of meat. Dietary utilization of BS could be suitable for developing low-input and low-emission feeding strategies to reduce food-feed competition and mitigate the environmental footprint of meat.

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SESSION 3

Animal Welfare

Monday 19 August 2024

THE EFFECT OF DIFFERENT CATTLE MARKETING ALTERNATIVES ON THE EATING QUALITY OF BEEF

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I. INTRODUCTION

Eating quality is becoming progressively important in the competitive protein marketplace as consumers are increasingly educated and meticulous in their selection [1] particularly with animal welfare credentials. Beef saleyards are a favoured marketing method in the Australian supply chain offering producers the ability to sell varying numbers and classes of cattle, contract agents to sell cattle on their behalf and providing competitive pricing [2]. However concerns regarding the impact of increased stressors and stimulation in the selling environment on eating quality and production has led to a higher proportion of cattle being sold through more direct pathways such as direct consignment [3]. Acute and chronic production stress has been found to adversely impact meat quality attributes including tenderness and ultimate pH [4, 5]. There is evidence that a resting or refeeding period prior to slaughter may assist is dissipating adverse eating quality effects caused by stress exposure throughout the supply chain [6]. The objective was to quantify the eating quality impact of alternative cattle marketing practices between four different saleyard treatments compared to direct consignment control cattle.

II. MATERIALS AND METHODS

The design utilised 5 treatment groups (n = 120) of mixed sex cattle of different breeds that were balanced within treatment from 4 different properties (2 supplied steers and 2 supplied heifers). The control treatment group (n = 24) were directly consigned to the abattoir (6 from each property), while the cattle for the other 4 treatment groups (24 from each property) were all penned with their property contemporary group during a livestock auction. The saleyard treatments were i) current Meat Standards Australia (MSA) saleyard pathway (36 hours from farm to knocking box, not mixed & water only), ii) 72 hours, iii) 7 days re-feeding and iv) 14 days re-feeding post sale. The 72 hour, 7 day and 14 day groups were mixed contemporary groups post-sale with access to total mixed ration and water. The impact of treatment on meat quality score (MQ4 = tenderness * 0.3, juiciness * 0.1, flavour * 0.3 and overall liking * 0.3) was measured using 400 untrained Australian consumers for grilled *M. longissimus lumborum* (Striploin) and *M. Semitendinosus* (eye round) aged for 7 days post-mortem. The effect of muscle, treatment group and their interaction on MQ4 score were analysed in a linear mixed effect model in R with animal within producer used as the random term.

III. RESULTS AND DISCUSSION

The 7 and 14 day refeeding treatments had adverse effects on MQ4 score by 6.4 and 5.94 points when compared to the directly consigned treatment ($P < 0.01$, Figure 1). The 48 hour treatment also tended to have a lower MQ4 score by 4.17 points ($P = 0.055$, Figure 1) compared to the direct consignment control. No significant differences in consumer eating quality factors were observed between the directly consigned group and the 72 hour treatment. Cut had a significant 16.19 point impact on MQ4 score with the striploin consistently higher than the eye round across all treatment groups. Hump height was the only carcass characteristic found to significantly impact consumer

sensory eating quality attributes. An 60mm increase in hump height from 40 to 100mm resulted in a 13 point reduction in MQ4 score in both the STR045 and EYE075 muscles.

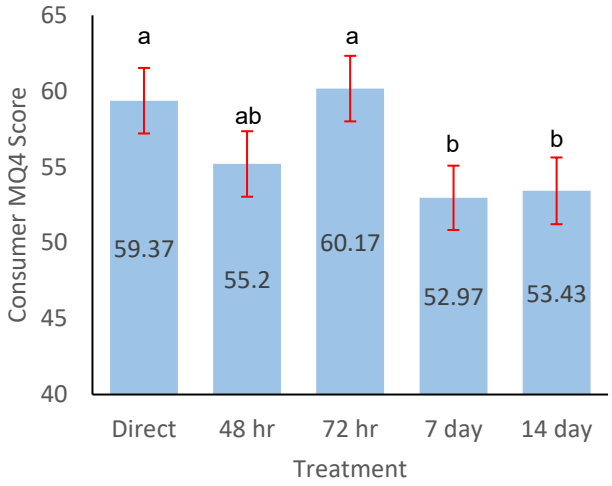

Figure 1. The estimated marginal means with 95% confidence intervals for eating quality score (MQ4) score for the M. *semitendinosus* (EYE075) and M. *longissimus lumborum* (STR045) combined for each marketing pathway. Different letters $^{(a,b)}$ indicate significant differences.

IV. CONCLUSION

This experiment identified that marketing method significantly impacted the intrinsic eating quality of beef for the consumer. Re-feeding cattle for a period of 7 or 14 days post saleyard exposure had a negative effect on eating quality. However extending the saleyard pathway out to 72 hours with access to feed did not negatively impact eating quality in this cohort. Further replication and research should aim to determine the extent of stress experienced by animals during the onsite re-feeding of cattle at saleyards to detect additional stressors during this time which may adversely impact eating quality.

ACKNOWLEDGEMENTS

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R-ZETA® ADDITIVE FATTENING DIETS IMPACT ON MEAT QUALITY CATTLE

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I. INTRODUCTION

Intensive cattle management systems encourage the inclusion of high amounts of cereal grains in the diet to increase energy input for improve animal performance. Nevertheless, since ruminants did not evolve by ingesting large amounts of non-structural carbohydrates, a series of metabolic disturbance may become a concern, and negatively impact rumen and animal health [1]. Self-feeders resulted in a homogeneous feeding pattern along the day and farmers used as an option to minimize digestive issues [2]. Large list of feed additives is added to beef cattle diets with expected benefits based on physical, chemical, and biological impacts. In addition to performance, it is important to evaluate the quality of the meat obtained. Thus, the aim of this study was to evaluate the meat quality from Hereford steers fattened with diets offered on self-feeders with intake regulators, salt (NaCl) or commercial compound additive r-zeta® (Los Vascos S.R.L).

II. MATERIALS AND METHODS

This research was carried out at the INTA Agricultural Experimental Station, located in the city of Concepción del Uruguay, Entre Ríos, Argentina (32°48'S, 58°34W). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the INTA Institutional Animal Care and Use Committee (N° 11). Forty-eight Hereford steers (7.4 to 8.7 months old and 220.5 ± 17.3 kg body weight) were allocated into two treatments according to the intake regulators: Control (diet formulated with 43% whole maize grain, 40% ground maize grain, 20% protein concentrate and 7% salt) and r-zeta® (pelletized balanced feed with inclusion of the aforementioned additive). R-zeta® is a multifactorial additive owned by Zorion US LLC and produced by Los Vascos SRL company in Coronda, Santa Fe province, Argentina. Its composition consists of specific flavorings for ruminants that regulate consumption, products that minimize thermal stress, enzymes, microorganisms that modulate the microbiota, liver protectors and anti-inflammatories. In all cases, diets were formulated to be isonitrogenous and isoenergetics and went offered in self-feeders. The animals were slaughtered when they reached 370 – 390 kg of BW. Half-carcasses were kept in cold chambers for 24 h (internal temperature <5 °C) and were moved to commercial packing house where samples were extracted and analyzed in the Meat Industry Lab (University of Entre Ríos). The chromatic meat and fat characterization were performed using Minolta colorimeter, operating in the CIE system (L*, a*, b*). Marbling was determined by visual comparison according to the standards of the Official United States Standards for Grades of Beef (USDA, 2018). Warner– Bratzler shear force (WB, N) was determined with a Stable Micro System texture analyser with a Warner–Bratzler cell. The average of six cylinders of 1.3 cm diameter was recorded with automatic temperature control and a temperature recorder with penetration sensor was used to follow the thermal process inside the samples. Thawing losses were calculated from the difference between initial fresh weight and weight after thawing (samples refrigerated for 24 h until reaching 4 °C in the center of the steak). Cooking losses (drip and evaporation) were determined by subtracting sample weight before and after cooking and expressed as percentage of the initial sample weight. Fat content of meat (on wet basis) was determined, in duplicate, by Soxhlet methodology using a 2055 Soltex and stove. Protein content of meat (on wet basis) was determined, in duplicate, by Kjeldahl methodology using a 2200 Kjeltec Auto Distillation-Foss Tecator equipment. Moisture was determined by rapid method 120 °C - 2 h, in quadruplicate. Statistical analysis was analyzed by ANOVA using software Infostat. Means were compared by the Tukey test (α= 0.05).

III. RESULTS AND DISCUSSION

The meat quality is presented in Table 1. No differences were observed meat color, marbling, tenderness, cooking losses or chemical composition and they are similar to report by other authors [3]. Only two variables differ, fat lightness and thawing losses. In the first case, the intensity of variation of Lightness will could not be detected by the consumers. And in the second, these results cannot explain of thawing losses in the Control group, considering both groups had the same chemical composition.

Table 1 – Quality of the *Longissimus dorsi* with additive r-zeta® on cattle fattening diets.

Traits	Control	r-zeta®	SEM	P value
L* meat	38.1	38.3	0.1	0.5970
a* meat	22.3	22.0	0.4	0.7507
b* meat	17.2	16.8	0.4	0.7001
L* fat	65.9 a	67.8 b	0.4	0.0014
a* fat	12.3	11.7	0.3	0.2954
b* fat	16.9	17.3	0.4	0.6642
Marbling[1]	2.8	2.4	0.2	0.4213
Tenderness (kgF^{-1})	2.9	2.8	0.1	0.5391
Thawing losses (%)	3.6 a	2.1 b	0.4	0.0333
Cooking losses (%)	21.0	22.1	0.8	0.5349
Humidity (%)	74.1	74.1	0.2	0.4074
Fat (%)	2.4	2.4	0.2	0.5898
Protein (%)	22.1	22.1	0.1	0.9943

a, b: means in the rows with a different letter indicate statistical differences ($P < 0.05$)
[1]: 1= standard. 2= select. 3= choice. 4= prime. 2: measured in the laboratory.

IV. CONCLUSION

The results demonstrate that feeding steers with diets that include the additive r-zeta® have not negative impact on the quality of the meat. Furthermore, some attributes such as fat luminosity and thawing losses improved.

ACKNOWLEDGEMENTS

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SESSION 4

Genetics and Physiology

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EARLY *POST-MORTEM* DISCRIMINATION BETWEEN BEEF TENDERNESS CLASSES IN FEEDLOT NELLORE BULLS USING MUSCLE LIPID BIOMARKERS

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I. INTRODUCTION

Tenderness is one of the most important traits related to meat quality. While consumers are able to discriminate among tenderness variations and are willing to pay a premium for tender beef [1], there is a significant inconsistency in this trait. Thus, omics approaches have been studied to better understand the causes of variability in tenderness and the biochemical mechanisms underlying tenderization. In a previous study, Antonelo et al. [2] observed muscle lipidome, mainly lipids from phosphatidylethanolamine class that are implicated in cellular apoptosis via mitochondrial permeability transition initiated by reactive oxygen species, affects the tenderness development. Therefore, in a dedicated study, we hypothesized that early *post-mortem* muscle lipidome segregates between tenderness classes. The aim of this study was to create an early *post-mortem* muscle lipid-based biomarker panel to discriminate between tender and tough beef.

II. MATERIALS AND METHODS

Carcasses from feedlot Nellore (*Bos indicus*) bulls (n = 100; from 20 to 24 mo age) were obtained at a commercial slaughterhouse. *Longissimus thoracis* (LT) muscle (11th rib level) samples were collected at 30 min *post-mortem* for further lipidome analysis. After 48 h of chilling (0°C to 2°C), the left side of each carcass was ribbed between the 12th and 13th ribs, and a 2.5-cm-thick LT sample of each carcass was obtained for Warner-Bratzler shear force (WBSF) analysis [3]. The WBSF values from all samples were utilized to identify the carcasses with the lowest (most tender; < 60 N) and highest (toughest; > 90 N) WBSF to create 2 beef tenderness classes (n = 10 per class; tender [average = 51.4 N; minimum = 39.7 N; maximum = 58.4 N; SD = 5.38] and tough [average = 100.9 N; minimum = 92.2 N; maximum = 116.5 N; SD = 8.1]). Each LT muscle sample (n = 10 per tenderness class) collected at 30 min *post-mortem* were ground in liquid nitrogen for lipid extraction using a method reported by Bligh and Dyer [4]. Targeted lipid profiling was performed using Multiple reaction monitoring (MRM)-profiling methods and instrumentation as reviewed by Xie et al. [5]. Further details about lipidome analysis have been described by Antonelo et al. [2]. Lipidome data were uploaded to Metaboanalyst 6.0 (https://www.metaboanalyst.ca/) and data were auto scaled prior to statistical and bioinformatics analyses. Principal component analysis (PCA) and hierarchical clustering heatmap were performed. Moreover, receiver operating characteristic (ROC) curve analysis was performed to create a predictive model for evaluating the performance of putative biomarkers for beef tenderness.

III. RESULTS AND DISCUSSION

Out of the 1366 MRMs scanned in a pooled sample for identifying the detectable lipids, 300 had intensities of at least 1.3-fold higher than the blank and were used for interrogating individual samples. The PCA (Figure 1A) and hierarchical clustering heatmap (Figure 1B) analyses revealed distinct

clusters between muscle lipidome from tender and tough beef. Based on the ROC curve (Figure 1C), a model was created to predict the beef tenderness, which obtained a maximum predictive accuracy of 73.3% using 50 putative biomarkers (Figure 1D), misclassifying only 4 out of the 20 samples.

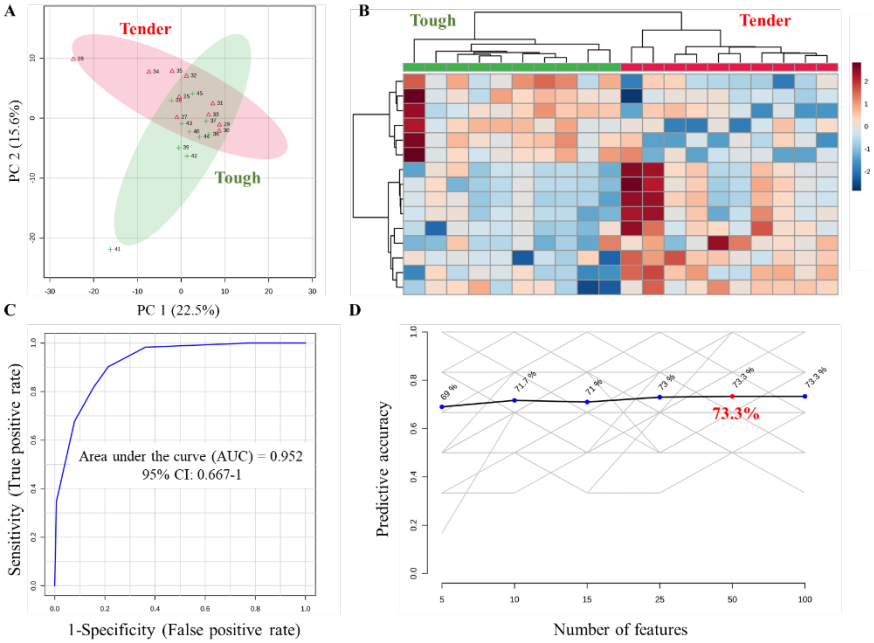

Figure 1. Muscle lipidome analysis from tender and tough beef. A) Principal component (PC) analysis; B) Top 15 hierarchical clustering heatmap analysis; C) ROC curve; D) Predictive accuracy of the model.

IV. CONCLUSION

Early *post-mortem* muscle lipidome from tender and tough beef has a discriminating profile, which allowed creating a preliminary predictive model to discriminate them right after animal's slaughter. Therefore, this early *post-mortem* muscle lipid-based biomarker panel can be used as a tool to assist and/or replace tenderness classification techniques performed in the late *post-mortem,* increasing the feasibility and reliability of the beef tenderness certification.

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Impact of visual dark-cutting severity and aging on the metabolomic profile of beef *longissimus lumborum*

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I. INTRODUCTION

Meat color profoundly influences consumers' decisions when purchasing beef products, serving as an indicator of freshness and quality [1]. However, persistence of myoglobin in the purple deoxymyoglobin state in dark-cutting beef presents significant challenges for the global meat industry due to its muscle darkening effect. The incidence of dark-cutting beef in the U.S. stands at 1.7% [2], while Canada and Australia have reported incidences at 2-2.5% [3] and 2.8% [4]. Limited studies have evaluated dark-cutting beef based on the severity of muscle darkening and the subsequent impact on color and metabolite profile throughout aging. We hypothesize that increasing dark-cutting severity will impact the beef metabolome. In addition, aging time will differently affect metabolite profiles. Therefore, the objective was to assess how varying degrees of dark-cutting severity influence the metabolic profile of beef *longissimus lumborum* at three different aging periods.

II. MATERIALS AND METHODS

At the time of grading, beef carcasses (n = 8/treatment) were selected from a commercial beef packing plant based on the visual degree of dark-cutting. These carcasses originated from *bos taurus* steers of primarily Angus genetics. These animals were sourced from commercial U.S. feedlots fed with a high-concentrate grain diet during the finishing period. After fabrication, beef strip loins (boneless, *longissimus lumborum*) were collected to represent a normal cherry-red colored control, one-half dark, and full dark-cutting. Loins were vacuum packaged and stored at 4 ± 2°C for further analysis. One 2.54 cm steak from the anterior end of each loin was cut at 48-60 h postmortem for metabolomics profiling, pH measurement, and bloom color analysis. The remaining portion of each loin was halved and assigned to either 21 or 39 d of wet aging in vacuum bags in dark storage at 4 ± 2°C. Following aging, one 2.54 cm steak was cut from the anterior end of each half loin for metabolomics and bloom color analysis. For bloom color analysis, steaks were horizontally bisected to expose the interior surface of the steak with one side allowed to bloom for 1 h at 4 ± 2°C. After 1 h of bloom, the color was evaluated using a HunterLab MiniScan EZ spectrophotometer. The remaining portion of each steak was used for quantitative metabolomics profiling via untargeted gas-chromatography-mass-spectrometry. Bloom and muscle pH were analyzed using the Glimmix procedure of SAS. Metaboanalyst 6.0 was used to analyze metabolite data. Significance was determined at an alpha value of 0.05 for all analyses.

III. RESULTS AND DISCUSSION

Full dark-cutting steaks had a greater ($P < 0.05$) pH early postmortem than normal and half dark-cutter steaks (pH: 5.51<5.91<6.39). Moreover, full dark-cutting steaks were darker in color and had lower bloom values early postmortem than full dark-cutting steaks from d 21 and 39 of aging. A total of 122 metabolites were observed across all treatment groups and the principal component analysis revealed distinct clusters in metabolite profiles between treatments. A reduction in differentially abundant metabolites was observed with aging time. Pairwise comparisons of the differentially abundant metabolites showed that half dark-cutting steaks exhibited the least changes compared with normal-beef, with 30, 7, and 7 differentially abundant metabolites on aging d 0, 21, and 39, respectively, while full dark-cutting steaks showed 56, 32, and 29 metabolites. Correspondingly, full dark-cutting steaks had a change in L^* value of 2.15% from d 0 to 39, while half dark-cutting steaks had a 6.15% change in L^* value indicating the potential role of metabolites on dark color. Metabolites differentially less abundant in full and half dark-cutting steaks on d 0 of aging were enriched for carbohydrate metabolism, while over-abundant metabolites were enriched in compensatory amino acid and nucleotide metabolism. By d 21 of aging, the differentially abundant metabolites in half steaks were all downregulated and involved in carbohydrate metabolism. Additionally, 50% of the differential metabolites observed on d 21 and 39 of aging in full dark-cutting stakes were enriched in amino acid metabolism, specifically alanine, aspartate, and glutamate metabolism, suggesting enhanced aging induced proteolysis in full dark compared with half dark steaks.

IV. CONCLUSION

In this study we show that varying degrees of dark-cutting severity in beef prompt distinct aging-related metabolite profiles compared to normal beef. Subtle changes in metabolites are noted at lower levels of muscle darkening and intensify at extreme levels. The observed differences in metabolite profiles throughout the aging periods relate to changes from carbohydrate metabolism towards compensatory energy pathways such as amino acid and nucleic acid metabolism. Moreover, the over-abundance of amino acid metabolites, particularly associated with alanine, aspartate, and glutamine metabolism in full dark-cutting beef at 21 and 39 d of aging, signifies enhanced muscle turn-over, emphasizing the impact of aging on muscle color.

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SESSION 5

Muscle biology & Meat quality

Tuesday 20 August 2024

DRY-AGING IMPACTS ON COLOR, FATTY ACID, AND LIPID OXIDATION OF STRIPLOIN FROM DAIRY CROSSBRED YEARLING AND 2-YEAR-OLD CATTLE

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I. INTRODUCTION

Producing beef from crossbred dairy surplus calves finished on pasture is an alternative farming strategy to reduce the environmental impacts of meat production and animal welfare concerns due to slaughtering bobby calves from the dairy industry [1]. Dry-aging is a value-adding strategy to produce high-quality beef with characteristic dry-aged meat flavor. It is unclear whether dry-aged meat from yearling animals have comparable quality and flavor intensity to older cattle. This study aimed to compare the impacts of in-bag dry-aging (BD) for 21 days on the color, fatty acids, and lipid oxidation of striploin from yearling and 2-year-old cattle.

II. MATERIALS AND METHODS

Twenty-four striploins (*M. longissimus lumborum*) were collected at 48 h post-mortem from dairy-beef crossbred yearling (~12 months old, n=12) and 2-year-old (n=12) cattle finished on pasture. Striploins were divided into four portions and assigned to BD (Tublin10®) for 0, 7, 14, and 21 days at 2 °C and 75% relative humidity. The weight losses during BD were recorded and pH was measured using a calibrated meat pH probe (Hanna 99,163 pH meter, USA). Instrumental color (CIE L*, a*, and b*) was measured on three random positions of freshly cut and bloomed (4 °C for 1 h) steaks using a calibrated Minolta Chroma Meter (CR-400, USA). Lipid oxidation level was estimated by the thiobarbituric acid reactive substances (TBARS) assay as described by Zhang [2]. TBARS results were presented as mg malondialdehyde (MDA) per kg dry matter to account for variations in moisture content. The fatty acids (FA) composition of aged samples was analyzed following a direct trans-methylation method by Agnew [3]. Major FA groups including straight-chain saturated FA (SC-SFA), branch-chain SFA (BC-SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), omega-3 FA (n-3), and omega-6 FA (n-6) were calculated and presented as the percentage in total FA. Data were analyzed using R software and “lme4” and “predictmeans” packages. Two-way ANOVA and Tukey’s honest significant difference ($P<0.05$) were used to determine any interactions between beef types and aging times.

III. RESULTS AND DISCUSSION

No interactions ($P>0.05$) were observed in most traits determined in this study between beef type and aging time except for TBARS ($P=0.006$) and BC-SFA ($P=0.029$). The pH values of striploins fluctuated throughout BD with significantly higher values observed in beef from yearlings than the 2-year-old after 0, 14, and 21 days of BD (Table 1). Meat color traits decreased while L* and hue angle tended to increase during BD in both meat types. Beef from yearling animals had darker, less red, and yellow color with lower color saturation index and hue angle following longer BD time compared to the older animals ($P<0.01$), probably due to the lower levels of myoglobin in beef from yearling animals and the higher weight loss during BD. However, there were no differences in hue angle after 21 days of BD. An increase of TBARS levels in samples from yearling animals ($P<0.0001$) was found after BD for 14 and 21 days with no significant changes from older animals during these times ($P=0.093$), indicating higher levels of lipid oxidation as BD progressed. Striploins from yearling animals had significantly lower percentages of MUFA, and SC-SFA, higher percentages of PUFA, n-3, and n-6, and similar BC-

SFA compared to older animals before BD. Most FA composition did not change after BD for 21 days compared to unaged equivalents (P>0.05) except for MUFA and BC-SFA. The decrease in BC-SFA (P=0.017) in beef from 2-year-old cattle may be due to the variations of fat content at different sample locations. The decrease of % MUFA (P=0.011) was only observed in yearling animals which could be linked to the increase of TBARS after 21 days of BD.

Table 1. Meat quality traits and lipid oxidation (TBARS, mg MDA/kg dry matter) of striploin from yearling and 2-year-old cattle during in-bag dry-aging for 21 days.

Traits	Yearling				2-year-old				Pr > F			
	Aging time (days)				Aging time (days)				Aging time (days)			
	0	7	14	21	0	7	14	21	0	7	14	21
% weight loss	-	17.53	27.26	29.70	-	12.90	19.89	22.90	-	***	***	***
pH	5.50	5.58	5.50	5.62	5.44	5.56	5.43	5.58	**	ns	**	*
*L** (lightness)	42.07	44.70	43.33	43.81	43.25	44.43	45.04	46.20	ns	ns	**	**
*a** (redness)	15.01	12.28	10.56	8.86	19.25	17.02	15.22	12.35	***	***	***	***
*b** (yellowness)	9.01	7.61	6.12	5.95	10.53	9.45	8.20	7.83	**	***	***	***
*C** (Chroma)	17.52	14.46	12.14	10.75	21.95	19.50	17.20	14.74	***	***	***	***
*h** (hue angle)	30.82	31.84	30.15	34.90	28.59	29.06	28.49	33.72	**	***	*	ns
TBARS	1.15	1.13	1.36	1.52	1.13	1.07	1.09	1.21	ns	ns	**	***

Levels of significance: * means <0.05; ** means <0.01; *** means <0.001. ns = not significant. Interaction between beef type and aging time was only observed for TBARS (P=0.006)

Table 2. Fatty acids (FA) composition (%) of striploin from yearling and 2-year-old cattle during in-bag dry-aging for 21 days.

	Yearling		2-year-old		Pr > F	
	Aging time (days)		Aging time (days)		Aging time (days)	
	0	21	0	21	0	21
Straight-chain Saturated FA	43.38	44.32	46.21	46.34	**	*
Branch-chain Saturated FA	1.38	1.45	1.31	1.18	ns	***
Monounsaturated FA	47.55	45.11	49.55	49.16	*	***
Polyunsaturated FA	7.68	9.13	2.93	3.32	***	***
Omega-3 FA	2.60	3.08	0.85	0.96	***	***
Omega-6 FA	3.49	4.24	1.36	1.60	***	***

Levels of significance: * means <0.05; ** means <0.01; *** means <0.001. ns = not significant. Interaction between beef type and aging time was only observed for Branch-chain Saturated FA (P=0.029)

IV. CONCLUSION

The dry-aging impacts on pH, color, and most FA groups were similar between yearling calves and 2-year-old cattle. Dry-aged beef from yearling calves had higher TBARS levels and % PUFA suggesting niche eating qualities and flavor profiles compared to mature cattle.

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PROTEOLYSIS IN VITRO REVEALS CALPAIN-1 ACTIVITY DURING THE BEEF MATURATION PROCESS

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I. **Introduction** - Significant efforts have been made to unravel the mechanisms underlying meat maturation and tenderization (Voges et al., 2007). While data supporting calpain-1 (CALPN1)-mediated proteolysis are robust, specific mechanisms responsible for controlling CALPN1 autolysis and its activity postmortem remain sketchy. Some debate regarding how long the protease remains active postmortem exist. Koohmaraie et al. (1988) suggest approximately 50% of the aging response or protease activity is achieved within 24 hrs postmortem in beef carcasses. While Boehm, Kendall, Thompson, & Goll (1998) found minimal degradation of desmin and titin within 24 hrs postmortem suggesting the bulk of proteolysis occurs after 24 hrs. To unravel these inconsistencies, we utilized an *in vitro* proteolysis system to address these outstanding questions and understand better CALPN1 activation during meat maturation.

II. **Materials and Methods** - Cross-bred yearling steers (n = 3) were harvested at the Virginia Tech Meat Science Research and Teaching Center using standard industry protocols. Bovine *longissimus thoracis* et *lumborum* (LTL) and the *extensor carpi radialis* (ER) samples were collected within 5 min post-exsanguination, deemed 0 d, and at 1, 2, 7, and 14 d postmortem. Myofibrils were purified from bovine *semitendinosus* (ST) 24 hrs post-exsanguination according to Weaver et al. (2007) and diluted to 50% with glycerin plus 0.1 MM phenylmethylsulfonyl fluoride for storage at -20°C. Glycerinated myofibrils were washed according to Huff-Lonergan et al. (1996) and Weaver et al. (2009). Protein concentration of preparations was determined and 4 mg/mL of isolated myofibrils were added to each digestion along with various treatments. To ensure the quality control of the myofibril preparation, three controls were utilized for each digestion. Control 1 contained only myofibrils; control 2 included myofibrils with 15 mM mercaptoethanol and 100 µM $CaCl_2$; and control 3 contained myofibrils with 15 mM mercaptoethanol, 100 µM $CaCl_2$, and CALPN1 (calpain-1, porcine erythrocyte, EC 3.4.22.17, Calbiochem, LaJolla, CA). Aliquots of all digestions were collected at 0, 2, 120, 480, and 1440 min. Control samples (0 min) were removed before addition of any treatments. Two min samples were taken immediately after the addition of treatments. Treatments included the addition of powdered LTL and ER muscles collected from carcasses at 0, 1, 2, 7, and 14 d. To stop reactions, aliquots of solubilization buffer (1:1) containing 8 M urea, 2 M thiourea, 3% (w/v) SDS, 75 mM dithiothreitol, 0.05 M Tris-HCl (pH 6.8) were added and used for subsequent SDS-PAGE and Western blotting protocols. Statistical analyses were performed using SAS Software (SAS 9.4, SAS Institute INC, Cary, NC, USA). Prior to data analyses, normality of residuals and homogeneity of variances were tested using the Shapiro-Wilk and Levene tests, respectively. Data were analyzed as a

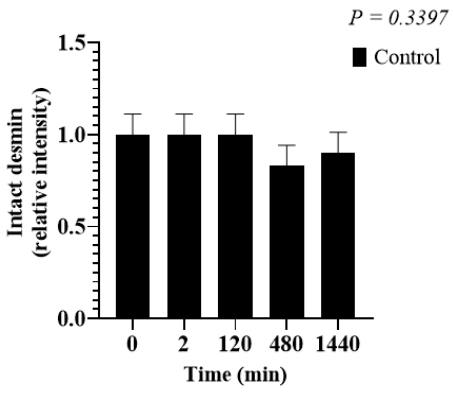

Figure 1: Mean percentage of desmin degradation.

completely randomized design, considering treatments as fixed effects and animals as random effects. Digestion data were analyzed as repeated measures. Differences were considered statistically significant when $P \leq 0.05$, or unless otherwise stated.

III. **Results and Discussion** - To ensure the validity and specificity of the model, we first showed isolated myofibrils were free of contaminating protease because myofibrils were used as a surrogate for assessing the amount and activity of endogenous proteases present in skeletal muscle. Figure 1 demonstrates that myofibrils experienced little desmin degradation after 1440 min in digestion buffer at room temperature. Next, we incorporated LTL muscle into myofibril digestions from carcasses aged 0, 1, 2, 7, and 14 d. Figure 2 shows that 0 d LTL and 1 d LTL had the least amount of desmin degradation, while 2, 7, and 14 d LTL had the greatest proteolysis *in vitro*. Because isolated myofibrils did not degrade in this system (Figure 1), any proteolysis came from muscle tissues derived from carcasses. To validate further our approach, we used two muscles known to differ in proteolysis postmortem. Specifically, we used the LTL, a muscle that undergoes significant proteolysis postmortem, and the ER that experiences negligible levels of proteolysis postmortem. Figure 3 shows minimal desmin degradation was observed when powdered ER was added to purified myofibrils regardless of aging time. These data argue the *in vitro* model containing purified myofibrils is a valuable approach to studying temporal changes in proteolysis as muscles age in beef carcasses.

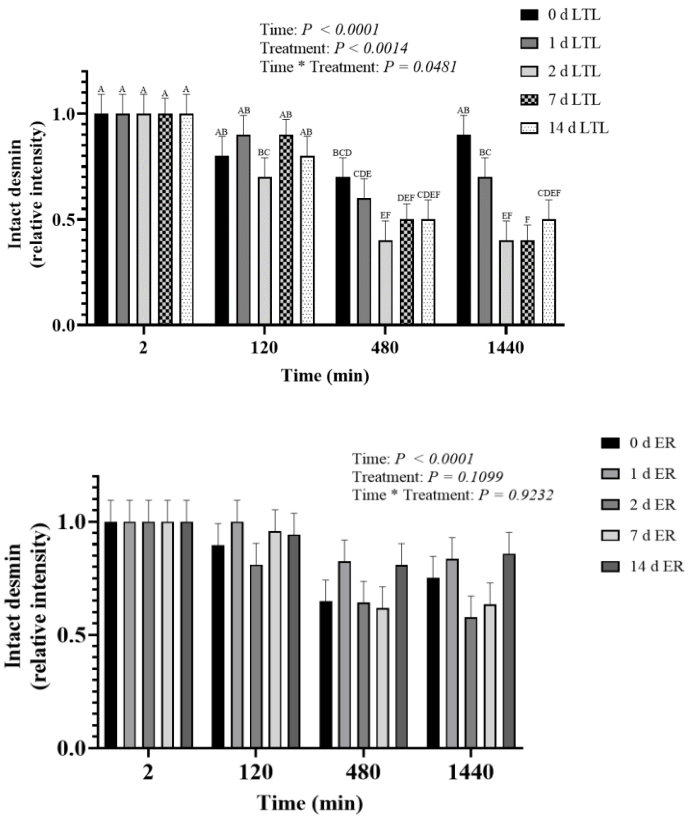

Figure 2: Mean percentage of desmin degradation

IV. **Conclusions** - These data show little 'active' protease exists in beef muscle prior to 2 d aging and argue this approach is a viable tool for studying how proteolysis is regulated postmortem.

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SESSION 6

Meat Safety

Tuesday 20 August 2024

Spray-on application of food cultures for safety and quality improvement of cooked, cured meat products

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I. INTRODUCTION

Application of lactic acid bacteria on cooked meat products after a final heat treatment aiming to improve shelf-life quality and/or safety is an emerging and promising technique for utilizing beneficial features of live cultures. Herein, it is demonstrated how spraying a defined level of *Latilactobacillus cuvartus* (SafePro® B-LC-48) cells as a post-pasteurization step contribute to inhibit growth of *Listeria monocytogenes* during shelf-life of hot-dog sausages with full level of nitrite or without nitrite added.
Another example of stabilization of product quality for cooked meat product is illustrated by spraying a *Lactococcus lactis* strain (Bactoferm® Rubis) during slicing/packaging. This prevents light-induced color fading because residual oxygen in packs efficiently is reduced below critical limit due to metabolic active bacteria. In general, such applications of lactic acid bacteria, represent clean label bio-solutions capable of replacing costly and energy requiring processing steps for a range of Ready-To-Eat products like cooked meat products, but also a technique to consider for plant-based meat alternatives and vegetable products.

II. MATERIALS AND METHODS

Part I: Hot-dog sausages (24% fat and 2.4% salt) were produced with two recipes: i) no nitrite added or ii) 108 ppm sodium nitrite added. A challenge test was conducted following ISO 20976-1 protocol with 3 batches included: control with 108 ppm nitrite; SafePro® B-LC-48 and 108 ppm nitrite and SafePro® B-LC-48 without nitrite.
A two strain *Listeria monocytogenes* cocktail was used. Inoculation level of *L. monocytogenes* targeted 10^2 cfu/g (SD 0.07 cfu/g), while the culture SafePro® B-LC-48 was applied by spraying an aqueous suspension with an inoculation level of 10^7 cfu/g to obtain a homogenous distribution on the meat product. Storage of samples was 20 days at 4°C followed by 40 days at 8°C.

Part II: A standard cooked, cured ham (2% salt and 120 ppm nitrite) was produced and sliced followed by packaging in modified atmosphere (60% CO_2 and 40% N_2). Samples were either control or sprayed with aqueous suspension of Bactoferm® Rubis on product surface during slicing targeting an inoculation level of 10^7 cfu/g. Initial storage period was at 4°C for 12 days, where packages were kept in the dark for initial 7 days before exposure to light (1200 lux) simulating retail display. After day 12 samples were kept at 8°C exposed to light. During storage level of residual oxygen and surface color parameters were measured.

III. RESULTS AND DISCUSSION

The challenge test with SafePro® B-LC-48 sprayed-on hot-dog sausages in vacuum packages show efficient inhibition of *L. monocytogenes*. Fig.1 shows evolution of the concentrations of lactic acid bacteria and *L. monocytogenes* during shelf-life at varying storage temperature. Fig. 2 shows residual oxygen levels in headspace of control and samples with Bactoferm® Rubis added over storage time. The insert plot shows differences in color parameters for the two samples observed after samples are exposed to light.

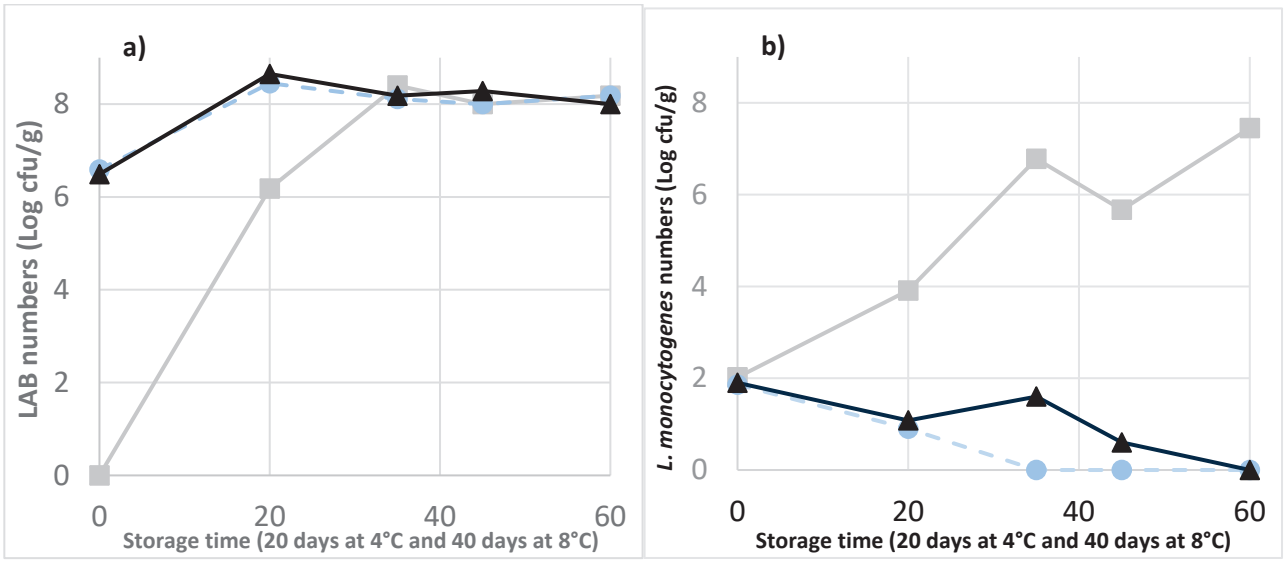

Figure 1. Challenge test in hot-dog sausages, evolution in lactic acid bacteria (LAB) concentration (a) or *L. monocytogenes* concentration (b) during the shelf-life. Symbols are: Control with 108 ppm nitrite ( ); B-LC-48 and 108 ppm nitrite (▲); and B-LC-48 without nitrite (●).

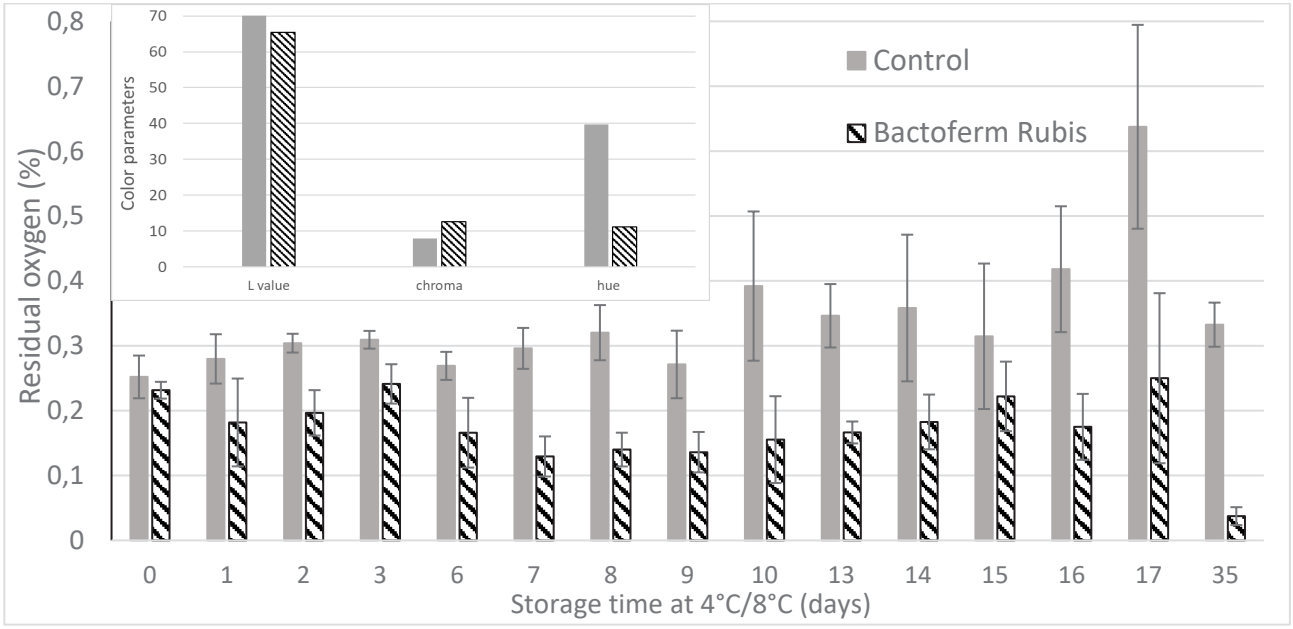

Figure 1. Part II trial with MAP cured cooked ham showing residual oxygen levels in headspace of samples with and without Rubis added. Insert plot shows color parameters for lightness, chroma and hue angel analyzed on day 7 after exposure of samples to light.

IV. CONCLUSION

Challenge test on emulsified sausages shows that SafePro® B-LC-48 culture helps protect against growth of *L. monocytogenes.* Samples sprayed with SafePro® B-LC-48 both with and without added 108 ppm nitrite have a growth potential $\delta = 0$, and concentrations of pathogen *L. monocytogenes* is in fact decreasing at all sampling times during the 60 days shelf-life. These findings hold good perspectives for food cultures in low or no nitrite meat products.

The test of Bactoferm® Rubis as a mean of protecting against photooxidation in cured meat products shows inoculated samples to have lower residual oxygen compared to control. This factor is preventing light-induced color fading when product is exposed to light as seen from higher chroma value and much lower hue angel indicating a more intense and redder color shade of product with Rubis added.

Effect of lactic acid, UV-C radiation and vacuum packaging on *Listeria monocytogenes*, *Salmonella* spp., *Pseudomonads* spp. and Lactic acid bacteria growth on raw chicken breasts.

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I. INTRODUCTION

Chicken meat is a very perishable food and a source of pathogenic bacteria such as *Listeria monocytogenes* and *Salmonella*. While packaging in modified atmosphere can extend its shelf life, it will not necessarily assure its safety, since some pathogens are able to grow before spoilage becomes evident [1]. Several research reports have addressed the efficacy of lactic acid (LA) for reducing microbial counts in meat and poultry [2]. More recently UV-C application has been shown to reduce L. monocytogenes and Salmonella on beef and meat products without affecting product quality. UV-C can also be applied after vacuum packaging [3, 4]. However, there are few studies on the combined effect of lactic acid, UV-C and vacuum packaging in chicken meat.
The aim of this work was to evaluate the effectiveness of lactic acid washing to reduce *L. monocytogenes*, *Salmonella enteritidis*, *Pseudomonads* spp. and lactic acid bacteria (LAB) on chicken meat vacuum packaged and stored at 4 °C.

II. MATERIALS AND METHODS

The effect of different doses of UV-C and lactic acid application, on *L. monocytogenes*, *S. enteritidis*, *Pseudomonads* spp. and lactic acid bacteria counts was analyzed in chicken breasts packaged under both aerobic and anaerobic conditions during 21 days.
A 2^2-factorial design with five central points was performed using Design-Expert 13.0 program. The independent variables were LA concentration between 0-5 % (m/v) and UV-C dose between 0-188 mJ/cm^2. The dependent variables were the counts expressed in log CFU/g of *L. monocytogenes*, *Salmonella*, *Pseudomonas* and LAB. For this purpose, 72 pieces of 10 grams were cut from freshly produced chicken breasts and inoculated with a mixed culture of *L. monocytogenes* ATCC 19111 and *Salmonella enteritidis* to reach a level of inoculation for both strains of 5.8 log CFU/g. After 10 minutes, inoculated samples were sprayed with 1.5 ml of LA according to the experimental design and placed in Cryovac T7335B bags. One half of the samples were vacuum sealed. Then, variable doses of UV-C were applied to the packaged meat according to the factorial design. Samples were analyzed at 0, 7, 14 and 21 days from the time of LA and UV-C application. Each piece of chicken breast was homogenized in a stomacher with peptone water and the appropriate dilutions were seeded on PALCAM agar plates with a selective supplement for PALCAM and incubated at 37 ºC for 48 hours for *L. monocytogenes*, on XLD agar and incubated at 37 ºC for 24 hours for *Salmonella*, in *Pseudomonas* Agar Base supplemented with cetrimide, fucidin and cephalosporin at 25 ºC for 48 hours for *Pseudomonas* and for LAB in MRS agar at 25 °C for 72 hours in anaerobiosis.

III. RESULTS AND DISCUSSION

Both lactic acid and UV-C significantly ($p<0.05$) reduce the counts of *L. monocytogenes* and PSE. While for *Salmonella* and LAB only the application of lactic acid was significant ($p<0.05$) to reduce the initial counts. The maximum level of reduction was achieved with 5 % LA and 188 mJ/cm^2 (Figure 1). The effect of 5 % LA and 188 mJ/cm^2 UV-C on growth inhibition for *Listeria* and *Salmonella* was observed during 21 days for both aerobic and vacuum packaged samples. While *Pseudomonads*

growth inhibition was only observed in vacuum packed samples. LAB counts increased after the 7th day regardless of the treatment (Figure 2)

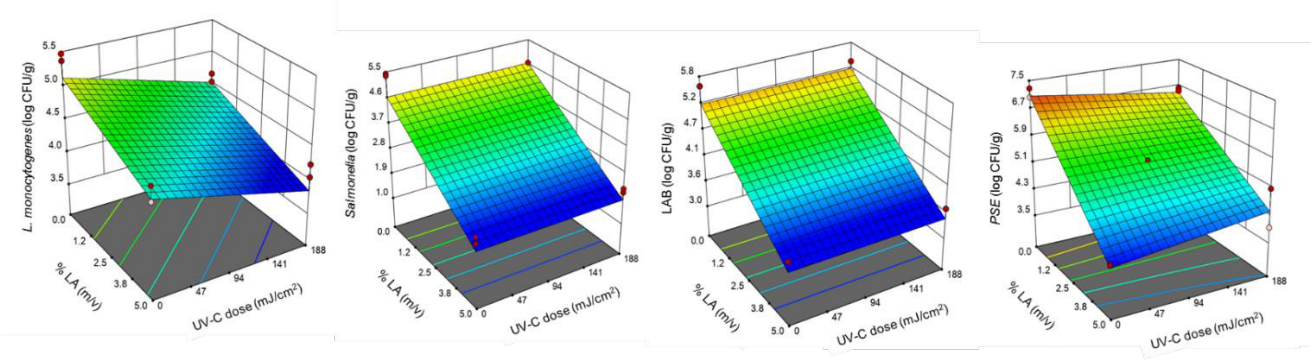

Figure 1. Effect of LA and UV-C on *L. monocytogenes*, *Salmonella*, PSE and LAB initial counts.

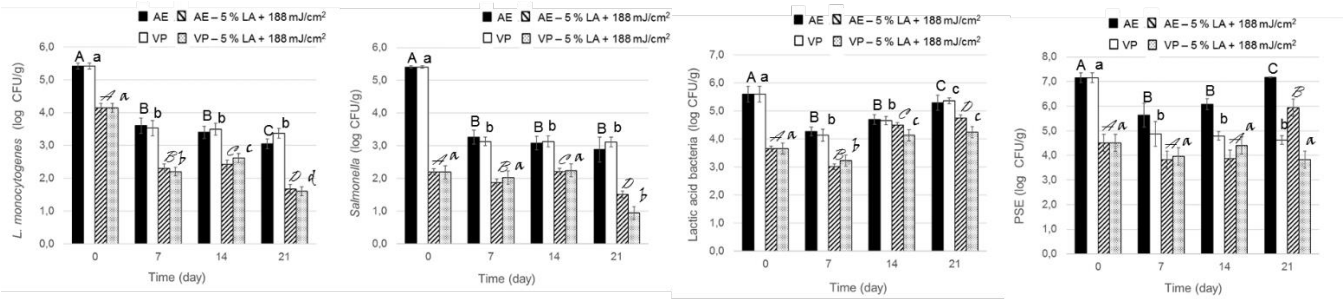

Figure 2. LA+UV-C and VP effect on *L. monocytogenes*, *Salmonella*, PSE and LAB counts for 21 days at 4 ºC.

IV. CONCLUSION

The application of 5 % LA + 188 mJ/cm^2 UV-C is useful to inhibit both *L. monocytogenes* and *Salmonella* spp. growth for 21 days at 4 ºC regardless of the packaging condition. 5 % LA + 188 mJ/cm^2 UV-C + VP is useful to control PSE up to 21 days at 4 ºC and up to 7 days for LAB. This strategy could contribute to improve the safety and extend the shelf life of refrigerated raw chicken breast.

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SESSION 7

Objective measurement of carcass and meat quality

Tuesday 20 August 2024

Beef fecal detection using a fluorescence multispectral camera system and deep learning object detection algorithm

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I. INTRODUCTION

Existing methods for fecal detection have primarily focused on visible feces. When visible excrement is detected, it is managed by cutting it out with a knife. However, fecal matter has the drawback of being diluted and difficult to detect after washing, as it often becomes less visible. Contamination by microorganisms such as *Salmonella spp.*, *E. coli*, *Campylobacter jejuni*, *Yersinia*, and *Listeria* can persist, highlighting the ongoing need for effective fecal detection (Gorji et al., 2022). Test methods such as ATP testing are used for quality control, but there are limitations in accurately pinpointing invisible contaminants. Specifically, in the context of beef, there is potential for detection using advanced fluorescent multispectral technology. This technology is effective because it allows the location of fecal matter to be visually communicated to the worker. This study aims to detect the fluorescence signals of minute contaminants using a multispectral imaging device that operates at wavelengths of 365 nm and 405 nm. The fecal matter on the beef carcasses exhibited red fluorescence. The research focused on developing an object detection algorithm for real-time detection using red fluorescence fecal images on beef carcasses.

II. MATERIALS AND METHODS

This study aimed to detect fecal matter on the surface of beef carcasses using fluorescence-based rapid detection equipment, specifically the CSI device (SafetySpect Inc, Grand Forks, ND, USA). For this research, LED sources emitting at wavelengths of 365 nm and 405 nm were chosen for their effectiveness in highlighting fecal matter, which appeared red under these conditions. Images were captured at a resolution of 768x1024 pixels. In total, 5807 images were included in the training set, 1243 images for validation set, and 1243 images were used in the test set for model evaluation. Object detection models were trained, including YOLOv8, YOLOv9, and EfficientDet. For hyperparameter tuning, the batch size for all models was set to 32, and each model was trained for 300 epochs. Model accuracy was evaluated using recall (%), precision (%), and F1 score (%). The study also calculated inference time, the time required to process one frame, indicating how quickly the model can be evaluated. Since fecal detection must be conducted in real-time, inference time is crucial for selecting the most suitable model. Thus, inference time was an essential factor in the model assessment. The study used Python version 3.8.9 and PyTorch version 1.12.1 for all analyses.

III. RESULTS AND DISCUSSION

Table 1 presents the results of various object detection models for identifying fecal matter on beef carcasses. In this study, the images were resized to fit the model requirements. Four models—YOLO v8-x, YOLO v8-n YOLO v9-e, and EfficientDet—were trained, and their results were compared. In terms of precision, YOLO v8-x demonstrated the highest performance. Conversely, EfficientDet exhibited the lowest precision. Regarding recall, EfficientDet achieved the highest recall rate, with YOLO v8-n also showing a high performance with a recall of 0.887. When evaluating the models based on the F1 score, YOLO v9-e attained the highest F1 score of 0.909. YOLO v8-n also produced a comparable result with an F1 score of 0.908. However, in comparing inference times, EfficientDet was the slowest at 0.407 seconds, indicating that the model's complexity hinders rapid evaluation. YOLO v8-n had the fastest inference time among the models tested. Although its F1 score was not the highest, it is considered the most suitable model for system implementation due to its quick inference time.

Table 1. Object detection model performance for detecting fecal on beef carcasses.

System type	Models	Precision	Recall	F1-score	Inference time
Object detection model performance	YOLO v8-x	0.952	0.862	0.905	0.024 sec
	YOLO v8-n	0.930	0.887	0.908	0.011 sec
	YOLO v9-e	0.938	0.882	0.909	0.044 sec
	EfficientDet	0.852	0.950	0.898	0.407 sec

YOLO v8 object detection result | YOLO v9 object detection result | EfficientDet result

Figure 1. Detection of fecal matter on beef carcasses and beef meat using deep learning models.

IV. CONCLUSION

This study was conducted to develop a model capable of rapidly detecting fecal matter on beef carcasses by utilizing deep learning-based object detection models. Notably, the highest performance was attained by YOLO v9-e, achieving an F1 score of 0.909. In selecting an efficient fecal detection model, YOLO v9-e demonstrated exceptional performance in terms of F1-score. At the same time, YOLO v8-n emerged as the most effective model, exhibiting both an appropriate F1-score and rapid inference time. Consequently, for systems necessitating real-time evaluation, YOLO v8-n is identified as the most suitable option. Nonetheless, further data accumulation may enhance the accuracy of these outcomes. This investigation affirms the viability of utilizing fluorescence images for fecal detection.

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THE USE OF DUAL-ENERGY X-RAY ABSORPTIOMETRY (DXA) FOR PREDICTING TOTAL AND INTRAMUSCULAR FAT IN PORK LOIN STEAKS

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I. INTRODUCTION

Many swine breeding companies are focused on developing animals with less subcutaneous fat and more intramuscular fat (IMF), aiming not only to make the meat tastier and more appealing but also to add value to the final product [4]. However, laboratory analysis to understand the composition of cuts, including fat analysis, is often laborious, expensive, and prone to errors [5]. To overcome these challenges and increase the speed and reliability of results, the use of modern technologies, such as dual-energy X-ray absorptiometry (DXA), has become an excellent tool to facilitate these analyses [3]. DXA is a non-invasive, easy-to-use and accurate technology that uses differential attenuation of X-rays to analyze key components of animal tissues such as bone, muscle and fat [4]. Therefore, in the present study, the hypothesis was raised that DXA can predict the amount of total fat and intramuscular fat in pork loin steaks, replacing conventional chemical analyses. Therefore, the objective of this study was to validate the fat results obtained by DXA, comparing them with standard chemical analyzes on pork loin steaks.

II. MATERIALS AND METHODS

Pork loin steaks (Longissimus lumborum) from approximately 140 animals sourced from commercial farms, with a thickness of approximately 2.5 cm, of undetermined sex, age, and genetics, were used. The steaks were identified, weighed, and dimensioned (width x length x height). These data was used as input into the GE Healthcare enCORE software, version 18, in "Small Animal" configuration mode [1]. Subsequently, the frozen steaks were scannedusing the DXA medical equipment (GE Healthcare, Lunar Prodigy Advance, USA), calibrated according to the manufacturer's protocol, in the Body Composition and Densitometry Laboratory at the Federal University of Viçosa. Twenty steaks were scanned per scan; and the software provided results for adipose tissue mass (g), lean tissue mass (g), total tissue mass (g), and fat content (%) per sample. Subcutaneous fat was then removed, and the steaks were weighed and scanned again on the DXA to evaluate only the intramuscular fat. Each steak sample was fully ground into meat grader, weighed, and subjected to lyophilization (Liobras, model LP510, São Carlos, SP, Brazil). After water removal by the lyophilization process, the samples were weighed again, frozen in liquid nitrogen, and subsequently ground in a stainless-steel ball mill (TECNAL, model R-TE-350, Piracicaba, SP, Brazil). Dry matter and fat content were determined using a sub-sample of the lyophilized samples. Chemical fat analysis was performed in duplicate using the Ankom XT4 filter bag and Ankom XT15 fat extractor (ANKOM Technology, Macedon, NY, USA), which uses petroleum ether as a solvent. The % chemical fat content was then calculated based on wet tissue. SAS 9.4 software (SAS – Statistical Analysis Systems Institute Inc., Cary, NC, USA) was used to perform general linear regression using the PROC REG procedure, following the model: $Y = \beta0 \pm \beta1X+e$ (where: Y is the observed fat represented by chemical analysis; β0 and β1 are regression components; x is the fat content predicted by DXA scan, and e represents random error). Equation performance was evaluated using the coefficient of determination (R^2) and root mean square error (RMSE) metrics.

III. RESULTS AND DISCUSSION

The regression equation for predicting total fat (steak with subcutaneous fat), had a better prediction accuracy (R^2= 0.48; RMSE = 4.24; P= <.0001) than the equation for pork loin steaks without subcutaneous fat (R^2 = 0.05; RMSE = 0.89; P= 0.0054) (Figure 1). Similar results were found by Nunes et al. (2023) [2],

who showed that marbling affects the accuracy of DXA in fat prediction. Prediction was more accurate in Angus steaks than in Nelore steaks, indicating better performance in samples with high fat content. In a literature review, Scholz et al. (2015) [3] compared the prediction accuracy of DXA in different studies. They noted that the relatively low absolute amount of fat leads to relatively larger prediction errors in percentage values for lean and fatty tissues. However, Soladoye et al. (2016) [4] evaluated the prediction by DXA of total dissected fat from carcasses of pigs of different breeds with varying fat contents, presenting equations with $R^2 > 0.80$. This demonstrates that DXA has high accuracy in assessing dissected fat composition. However, the accuracy and precision of fat prediction vary when DXA performance is assessed in low and high-fat content groups, showing better performance in high-fat steaks [2]. However, DXA is a useful tool in animal research, but further studies are needed to assess its ability to predict IMF across a wide range of percentages, aiming to develop a robust regression model regardless of fat content.

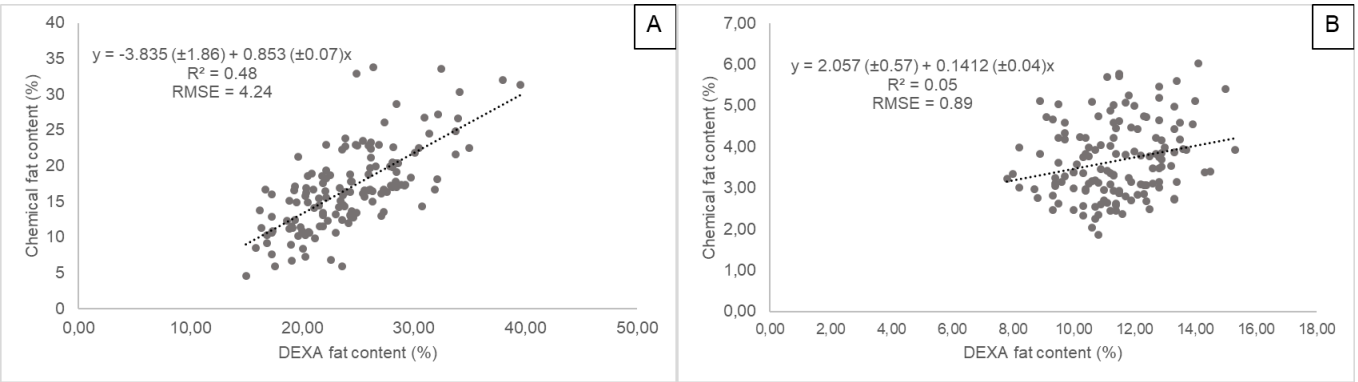

Figure 1: Linear regression between the fat content values predicted by DXA, on the x-axis, and the corresponding chemical analysis, on the y-axis, for steak with subcutaneous fat (A) and steak without subcutaneous fat groups. Estimated parameters such as coefficients of determination (R^2) and root mean square error (RMSE) are presented in the graphs.

IV. CONCLUSION

This study shows that DXA can predict total fat content in pork steaks, but not the intramuscular fat content. Its accuracy varies between low- and high-fat groups, with it performing best in high-fat steaks, and it can be useful for “precision labeling” of pork loins, allowing consumers to monitor the amount of fat in their packaged steaks.

ACKNOWLEDGEMENTS

Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), #RED-00172-22 and #BPD-00648-22; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Ciência e Tecnologia de Ciência Animal (INCT-CA), #465377/2014-9.

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SESSION 9

Meat Products Development

Thursday 22 August 2024

Effect of smoking on the texture of different casing-types of frankfurters

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I. INTRODUCTION

Natural casings are considered the best because of their unique properties such as bite, kink, curvature and snap. Not all of these properties such as flexibility, compressive strength, smoke absorption and permeability can be replicated by synthetic casings. This is the reason why natural casings are often considered the best option for sausage casings and are expected to remain the preferred choice for a lot of sausages (Suurs et al. [1]). Alternatively, synthetically produced collagen is used, which accounts for 80% of all edible casings, and has these advantages of uniformity in size, strength and flexibility under different processing conditions, as well as better consistency of net product weight, casing's elasticity, higher hygiene status, and therefore longer shelf life. Another alternative is cellulose casings, which have advantages in terms of strength, ease of processing and shelf life. As cooked sausages, such as frankfurters, form a stable, self-supporting gel when heated and filled in casings, casings do not always have to act as mold holders. These casings allow small molecules such as water and small organic molecules found in wood smoke to pass through. Larger molecules, such as fat and protein, are largely retained and can withstand the conditions in a smokehouse with high relative humidity (Barbut [2]).

II. MATERIALS AND METHODS

For the production of the Frankfurter Sausages, following materials were used: 30 % RIII beef meat, 30 % SIII pork meat, 18 % SIX pork fat, 22 % ice, 1.8% nitrite curing salt, 0.2% phosphate, 0.05% ascorbic acid, 0.5% spice mixture, 0.2% dextrose, 1% fresh onion, 0.1% garlic paste. Once the sausage batter has been produced, it is filled into sheep casings (22 mm), collagen casings (21 mm) or cellulose casings (19 mm). For the smoking of the Frankfurters, five different smoke generator temperatures and smoking times were conducted per casing type. Those were: 300 °C for 10 min, 450 °C for 7 min, 600 °C for 6 min, 750 °C for 4 min and 900 °C for 3 min. During the smoking process, beech (diameter of 6 – 12 mm) were used to produce the smoke. The followed cooking step was at 75 °C for 20 min which led to an internal temperature of 72 °C.

To assess the tenderness of the Frankfurters, the Warner Bratzler shear force measurement was performed. 12 measurements for each sausage type were conducted using four sausages per type in total, all at 7 °C. To perform the shear force measurement, the sausage sample was positioned in the center, directly beneath a V-shaped blade. Once the measurement started, the blade steadily moved towards the sample with a speed of 1.5 mm/s and applying pressure until the sausage reached its breaking point. Throughout this process, the force applied by the blade was continuously measured.

The tensile strength was determined as the maximum stress until the casing broke (Suurs et al. [1]). Only the sausages with natural casing or collagen casing were used for this test. A total of 12 samples per sausage type were prepared by removing all residues from the sausage meat with a razor blade and roughly removing it from the casing. This led to thickness of about 0,5 mm. The casings were cut into 7 cm x 4 cm pieces and packed in plastic bags under a controlled atmosphere and stored overnight at 5 °C. To perform the measurement, the sample was clamped between the fixtures at a distance of 4.2 cm. The clamps were moved in the opposite direction at a speed of 0.2 mm/s until the break was reached and the force was measured continuously

III. RESULTS AND DISCUSSION

The findings in Figure 1 (left) indicate that the peeled cellulose casing samples exhibited the lowest maximum force with a range of 23.3 ± 0.8 N to 27.5 ± 1.0 N at 300 °C and 750 °C, respectively. These results are statistically significant and demonstrate that the peeled cellulose casing samples differ

significantly from the other casing type samples and are, thus, more tender. The removal of the casing, which is responsible for the hardness of the other samples, is the main reason for this difference. This is, on the one hand, due to the drying out of the surface. On the other hand, the reduction of myofibrillar and sarcoplasmic protein nitrogen and increase in stromal protein nitrogen leads to cross-linking of surface proteins and therefore a firmer outside of the smoked product (Maga [3]). Overall, the collagen and natural casings yielded similar results with notable deviations at 450 °C and 900 °C. These findings confirm that frankfurters in peeled cellulose casing exhibit greater tenderness despite the smoking temperature. In contrast, frankfurters smoked in ESC and NC at 300 °C, 600 °C or 750 °C display insignificant differences in tenderness.

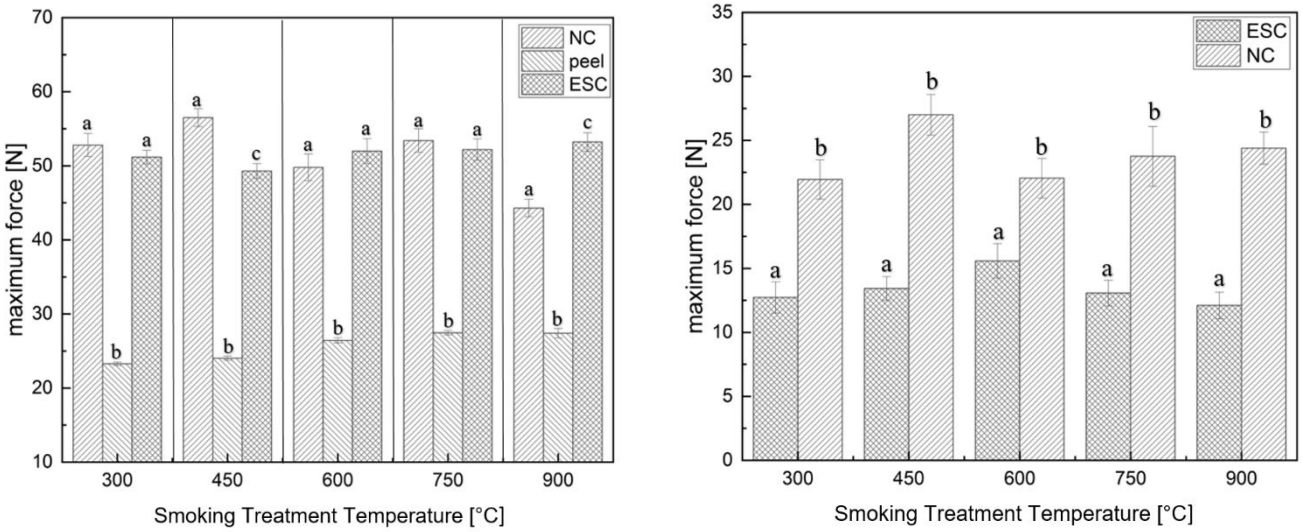

Figure 1: Maximum force [N] of the Warner Bratzler analysis of all casing types and temperatures (left) and of the Tensile strength test of collagen (ESC) and natural casing (NC) and all smoking temperatures.

The statistical analysis of the data displayed in Figure 1 (right) indicate that NC and ESC frankfurters are significantly different from each other, regardless of the smoking temperature. While the values for the collagen casings range from 12.1 ± 1.0 N (900 °C) to 15.6 ± 1.3 N (750 °C), the natural casing samples endured a higher force between 21.9 ± 1.5 N (300 °C) to 27.0 ± 1.6 N (450 °C). These results suggest that the hardness of the casings does not depend on the smoking temperature but rather on the type of casing that is being used.

IV. CONCLUSION

Different experiments were conducted to investigate the characteristics of frankfurters produced with natural casings, collagen casings, and peeled cellulose casing. The texture analysis clearly showed a more tender first bite in peeled cellulose casing frankfurters when compared to those encased in natural or collagen casings. Furthermore, the results demonstrated the higher tensile resistance of natural casings in comparison to collagen casings.

ACKNOWLEDGEMENTS
This IGF Project of the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn) was supported via AiF [21343N] within the program for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi).

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Fermentation of pea and soy protein with *Lactilactobacillus plantarum* improves flavor of plant-based burger patties.

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I. INTRODUCTION

First time buyers of Plant Based Meat Alternatives (PBMA) have recently been found to decline over a 2-year period [1], which is in line with a general stagnation observed in global sales of PBMA products. There can be many reasons for consumers not buying or repeat buying PBMA products, e.g. taste issues, cost price, ultra-processed, too many ingredients or quality aspects like texture and appearance. Flaws in taste and flavor of PBMA products are listed as main reason for not buying or rebuying by approx. 50% of US consumers [2].
The aim is to investigate if fermentation of soy or pea texturized protein with *L. plantarum* (MCX-2401) before preparation of PBMA burgers improves flavor and modifies volatiles detected.

II. MATERIALS AND METHODS

Samples with fermented soy or pea texturized protein are prepared and two controls with non-fermented plant proteins are used as references. Fermentation of texturized protein is carried by adding culture *L. plantarum* into water followed by mixing of culture suspension into dry texturized proteins (2.5:1 ratio) targeting an inoculation level of 4×10^6 cfu/g. The hydrated texturized proteins are incubated at 24°C or 30°C and then cooled to 5°C when pH reaches 5.5-5.6 in the fermented protein. Dough for burgers is made from water, mix of (fermented) texturized protein and protein isolate, while the fat part is vegetable oil and coconut fat. Minor components added are citrus fiber, lactate, tomato puree, methylcellulose, red beet color, spices, salt, and emulsifier. Preparation is by pre-grinding (6/3 mm) of fermented/hydrated texturized protein, which is mixed with dry ingredients suspended in remaining water, and frozen pre-grinding (6/3 mm) coconut fat/oils, while keeping temperature at 0°C. Burger patties are shaped each weighing 113 g.
In trial 1 a descriptive sensory test with 14 assessors is conducted using 13 pre-defined descriptors with Rate-All-That-Applies (RATA) to evaluate 4 fried plant-based burgers patties. In trial 2 semi-quantitative GC-MS headspace analysis of volatiles is performed on 3 soy-based burgers. Texturized protein is analyzed for Lactic acid bacteria (LAB) by plating on MRS agar. Final pH value is measured in plant burgers for 4 samples with or without fermentation of 2 texturized plant protein types.

III. RESULTS AND DISCUSSION

The fermentation step resulted in an increase in LAB concentration of more than 2 log cfu/g compared to initial inoculation level at 4×10^6 cfu/g. This also brings a drop in pH which impacted final pH value of the dough, where soy control has pH 6.6 while dough with fermented texturized soy protein has pH 5.5. For pea protein samples the respective pH values of control and pre-fermented samples are at 6.0 and 5.6, respectively.
From the descriptive sensory data submitted to multivariate analysis in Fig 1, samples with fermented texturized pea or soy protein are evaluated quite similar compared to the two non-fermented control samples. Also, the average scores for descriptors show that umami and meaty are rated significantly higher in fermented samples compared to controls. In contrast, for beany attribute the control samples both receive significant higher scores compared to both pre-fermented soy and fermented pea protein samples.

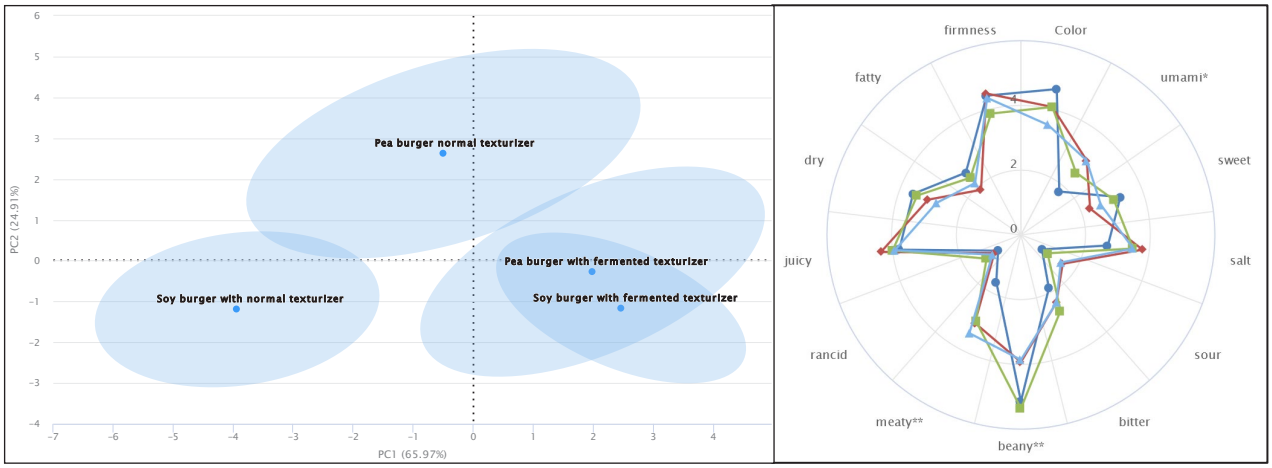

Figure 1. Sensory evaluation of fried plant-based burgers in trial 1 showing scores plot (a) from principal component analysis and spider plot (b) with average scores for 13 descriptors in fermented soy (-◆-), fermented pea (-▲-), control soy (-●-) and control pea (-■-). Descriptors marked with * or ** indicate statistically significant difference on $p<0.05$ or $p<0.01$ confidence level, respectively.

In Fig 2 lipid-derived carbonyls, pentanal, hexanal and heptanal associated with beany flavor notes, are reduced to 1/3 on average compared to amount in non-fermented texturized soy protein control.

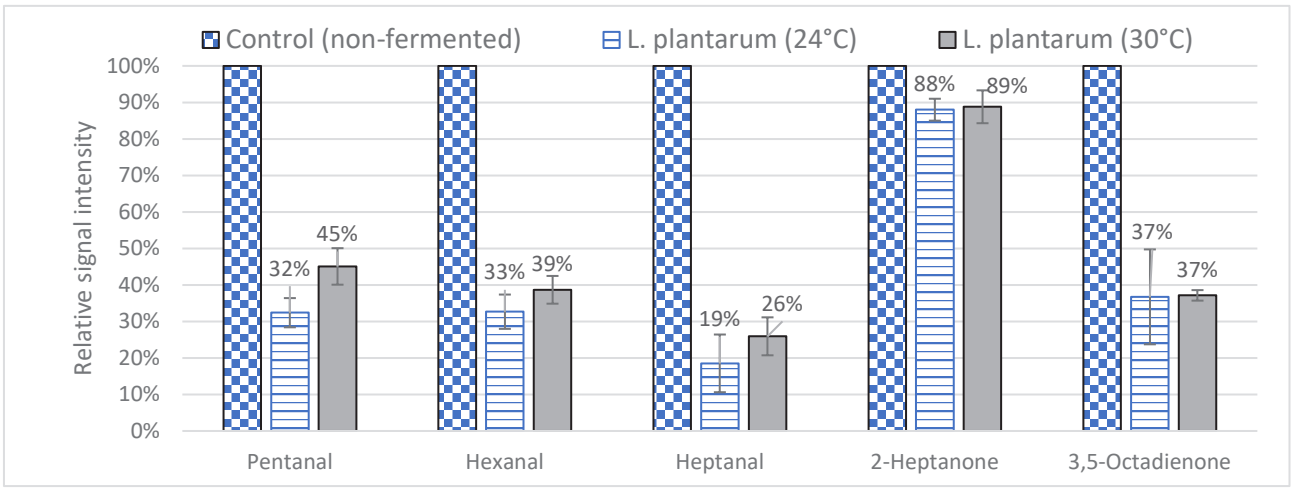

Figure 2. Analysis of selected volatiles, carbonyls and ketones, in trial 2 on fried soy-based burger patties. Signal intensity for non-fermented control is set as 100% and reduction in signal intensities for fermented samples shown as percentage.

IV. CONCLUSION

Fermentation of texturized soy or pea protein used in plant-based burgers can significantly improve taste and flavor by increasing meaty and umami ratings, while unpleasant beany note is reduced. Thus, fermentation of plant-based proteins is potentially a natural and traditional solution to address before-mentioned challenges regarding poor taste and flavor in PBMA products.

A substantial reduction in volatiles like pentanal, hexanal and heptanal due to fermentation is most likely accountable for observed decline in beany and corresponding increase in meaty and umami ratings.

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SESSION 10

Meat Products Stability

Thursday 22 August 2024

Influence of air contact and pre-packaging treatment on the color stability of vacuum packaged beef

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I. INTRODUCTION

Today the most used aging method for meat is wet aging [1]. In this aging method, the meat is vacuum packaged and stored at low temperatures for a certain time [1]. Endogenous enzymes tenderize the meat, while spoilage, pathogen growth, and drying loss are minimized by the protective vacuum packaging [1]. As the color of meat is an important quality indicator for the customers, it is of highest importance, that the meat keeps its color during storage [2,3]. To test the influence of different (mis)treatments of the meat such as mechanical damage and extended storage at air before packaging, as well as different extents of vacuum, meat samples are packaged, and their spectral data is measured over the storage time. From the spectral data, the change of myoglobin redox forms is calculated to obtain information regarding discoloration processes. The experiment aims to determine the extent of the impact of the factors and whether production processes require optimization in certain areas.

II. MATERIALS AND METHODS

Vacuum packaged beef (from 3 young bulls) shoulder parts obtained frozen at -18 °C and thawed for 2 days at 2 °C. Then the packages are opened, and connective tissue and fat are manually removed using a knife. Afterwards, the samples are treated and packaged in polyamide/polyethylene foil as shown in Tab. 1. A slice of each animal underwent the different treatments to obtain biological replicates. The technical replicates were achieved by measuring each sample in triplicate.

Table 1 – Treatment methods and packaging atmospheres of the beef samples.

Atmosphere	Composition/Treatment
High Vacuum (HV)	15 mbar residue pressure
High Vacuum punctured (HV punctured)	15 mbar residue pressure, samples are punctured with a meat tenderizer before packaging
High Vacuum 2 hours of air contact (HV 2h Air)	15 mbar residue pressure, samples are in air contact for 2 hours before packaging
Low Vacuum (LV)	60 mbar residue pressure
Synthetic air (Air)	20 % oxygen, 80 % nitrogen

After packaging, the spectra of the samples were measured in the packaging over a time period of 14 days using a HunterLab UltraScan VIS 1091 Spectrophotometer. Between the measurements, the samples are stored dark at 2 °C. The sample packages and measurements both were performed in duplicate. The spectral data was used to calculate the myoglobin redox forms according to Li et al. [2]

III. RESULTS AND DISCUSSION

The calculated myoglobin redox forms can be seen in Fig. 1. The oxymyoglobin (OMb) levels show similar and constant values of all vacuum packaged samples between 0.3 and 0.4. An exception are the samples that were exposed to air before packaging and reached a value of 0.54, which is even higher than the value of the air-packed sample that was used as a reference (0.49). After one day of storage, the OMb levels of the HV 2h Air samples dropped to the level of the other vacuum packaged

samples, while the air packaged samples obtained their higher OMb level. This behavior can be explained by oxygen consumption due to mitochondrial respiration, leading to the deoxygenation of the oxymyoglobin [3]. The deoxymyoglobin (DMb) levels behave similar to all vacuum packaged samples except the HV 2h Air samples. They start at DMb levels around 0.75 increase slightly over the first day and then decline to final levels around 0.67. The HV 2h Air and Air samples start at lower DMb levels around 0.67 with the air sample constantly decreasing.

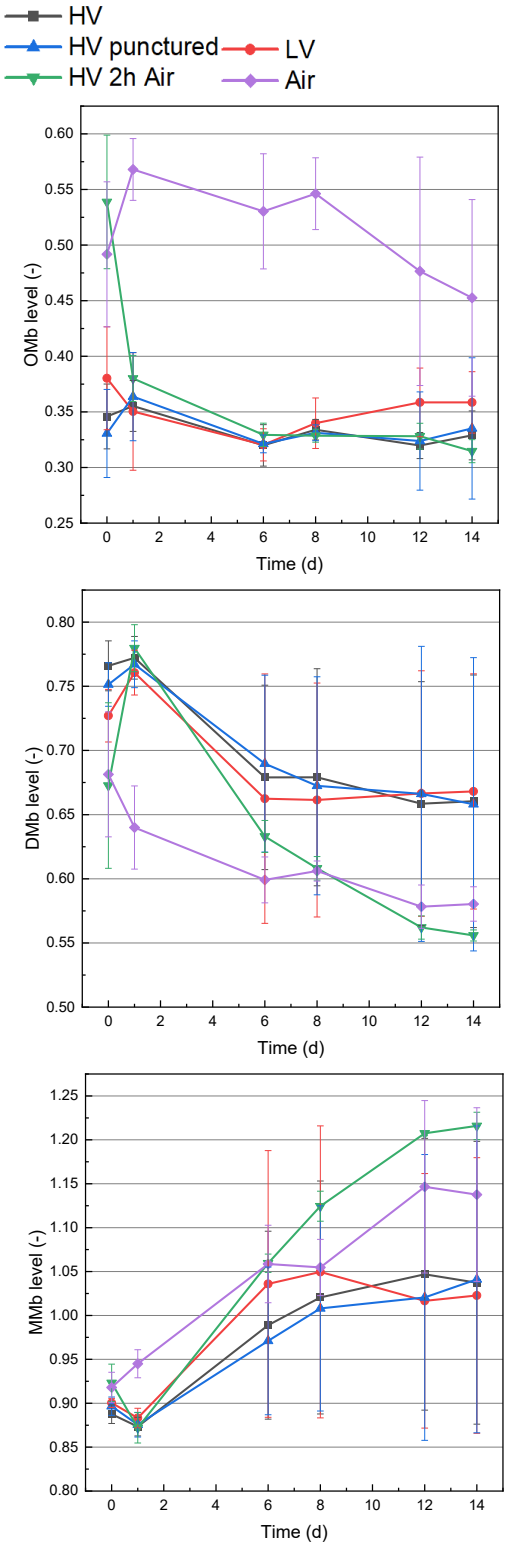

Figure 1. Oxy- (A), deoxy- (B), and metmyoglobin (C) levels of beef samples during storage.

The HV 2h Air samples show a strong increase in DMb levels over the first day in connection to the OMb decrease due to mitochondrial respiration [3]. Afterwards the DMb levels decrease strongly to slightly above 0.55 at day 14. This observation can be explained with the strong increase in metmyoglobin (MMb) levels of the samples. While all samples start at similar MMb levels around 0.9 the HV 2h Air samples show the highest final values of 1.22. The second highest MMb level is reached by the air packaged samples (1.12). The remaining vacuum packaged samples reach final values between 1.00 and 1.05. As the OMb level of these vacuum-packed samples is relatively stable, the decrease in DMb level and the corresponding increase in MMb level can be explained by MMb formation during storage, which leads to discoloration of the meat.

IV. CONCLUSION

The extent of vacuum applied in this experiment or mechanical damage did not influence the discoloration strongly. The prolonged air contact pre-packaging however, negatively influenced the meat color leading to higher MMb levels than simple air storage. These findings suggest that for optimal color stability, meat should be packaged as quickly as possible.

ACKNOWLEDGEMENTS

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Addition of freeze-dried beef exudate alters volatile flavor profile of cooked ground beef patties

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I. INTRODUCTION

Beef flavor is composed of taste and aroma. Non-volatile taste contributing compounds include sugars, free-amino acids, small peptides, nucleotides, and others. Reducing sugars and free-amino acids, may also contribute to development of volatile beef aroma through the Maillard reaction. Water soluble heme iron containing myoglobin also contributes to beef taste [1]. Model studies have demonstrated hemoglobin and free iron contribute to beef aroma development [2]. Commonly beef is held in packaging under refrigerated temperatures. During this time water-based exudate is released in packaging. Meat exudate is rich in water-soluble compounds [3]. We hypothesized that meat exudate could be utilized to influence beef flavor. Therefore, the objective of this study was to determine if the addition of freeze-dried beef exudate to ground beef impacts volatile flavor compounds.

II. MATERIALS AND METHODS

Beef exudate was collected from individually vacuum packaged *M. Longissimus lumborum* and M. *Gluteus medius* beef steaks following 28 d of refrigerated (4°C) storage. Approximately 500 mL of exudate was comingled from 96 steaks. After collection, exudate was frozen and stored at -80 °C in 50 mL conical vials. Conical vials were thawed at 4 °C for 24 hours before being freeze dried (VirTis Genesis 25L SQ Super ES-55 pilot lyophilizer, SP Scientific, Gardiner, NY). Dried exudate was added to 80:20 ground beef at 1 and 3%, by weight. A control treatment received no addition. Additions were made to to reach a final weight of 100 g before using a hand press to form beef patties. Nine patties were formulated per treatment. Patties were cooked on a cast-iron skillet heated to a surface temperature of 200 ± 10 ◦C to an internal temperature of 71 ◦C. Volatiles were collected by solid phase microextraction (SPME) using an 85 µm film thickness carboxen polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA, USA). After extraction, volatiles were separated by gas chromatography using a VF-5ms capillary column (30 m × 0.25 mm × 1 µm; Agilent J&W GC columns, Santa Clara, CA, USA). Compounds were measured by quadrupole mass spectrometer (5977A, Agilent, Santa Clara, CA, USA) via electron ionization at 70 eV. External analytical grade standards (Sigma-Aldrich, St. Louis, MO, USA) were used to confirm compound identities through retention time and the fragmentation patterns. Quantitation of volatile compounds (ng per gram of sample) was conducted using the internal standard and a 5-level calibration curve.Statistical analyses were conducted using the PROC GLIMMIX procedure of SAS (v. 9.4, Cary, NC, USA). The Kenward–Roger adjustment was used to estimate denominator degrees of freedom. Least squares means were separated using the PDIFF function. Alpha was predeterimend to be 0.05.

III. RESULTS AND DISCUSSION

Among measured compounds 24 differed due to treatment ($P \leq 0.046$, Table 1). Strecker aldehydes, Methional, Benzaldehyde, and Phenylacetaldehyde were greater in the 3% treatment compared to all others ($P < 0.05$). Therefore, 3% inclusion of dried exudate into beef patties may provide additional free-amino acid precursors to participate in the Maillard reaction. Conversely, Acetoin was greater in control samples compared to 3% ($P < 0.05$). Acetoin is an odor active intermediate of the Maillard reaction which may generate Strecker aldehydes [4]. Lower Acetoin with 3% inclusion of dried exudate

may be due to enhanced participation of Acetoin in the Strecker degradation pathway. Among lipid derived volatile compounds, Nonanoic acid, Decanal, 2,4-Decadienal, 2-Undecenal, and Decane were greater in the 3% treatment compared 1% and control treatments ($P < 0.05$). Conversely, Methyl butyrate, Methyl Heptanoate, Pentanal, 2-Heptanone, and 2-Pentylfuran were each greater in control compared to all others ($P < 0.05$). Methyl octanoate and Hexanal were each greater in control compared with 3% ($P < 0.05$). Lipid degredation products may participate in the Maillard reaction [5]. Therefore, inclusion of dried exudate may influence interactions among Maillard intermediates and lipid degradation compounds.

Table 1 – LS means of volatile compounds (ng/g) from cooked ground beef patties (control) or ground beef patties contining 1 or 3% freeze-dried beef exudate by weight.

Volatile Compound	Control	1%	3%	SEM[1]	*P-value*
Benzaldehyde	7.23^{b}	8.15^{b}	9.75^{a}	0.44	<0.001
Methional	0.64^{b}	0.77^{b}	1.32^{a}	0.11	<0.001
Phenylacetaldehyde	4.82^{b}	5.11^{b}	6.59^{a}	0.31	<0.001
Acetoin	21.0^{a}	19.5^{ab}	15.3^{b}	1.32	0.015
Carbon Disulfide	24.7^{b}	171.4^{a}	189.4^{a}	33.3	0.003
Furfuryl Sulfide	42.21^{a}	12.54^{ab}	8.88^{b}	6.55	0.027
1-Pentanol	6.57^{a}	5.87^{b}	5.93^{b}	0.21	0.04
2-Heptanone	4.84^{a}	4.74^{b}	4.68^{b}	0.03	0.003
2-Pentyl Furan	4.73^{a}	4.60^{b}	4.61^{b}	0.04	0.036
2-Undecenal	1.56^{b}	1.57^{b}	1.97^{a}	0.07	<0.001
2,3-Pentanedione	6.81^{b}	7.48^{ab}	7.73^{a}	0.23	0.023
2,4-Decadienal	0.45^{b}	0.46^{b}	0.55^{a}	0.02	<0.001
D-Limonene	4.40^{a}	4.39^{ab}	4.38^{b}	0.003	0.013
Pentanal	11.8^{a}	9.11^{b}	8.32^{b}	0.78	0.009
Hexanal	42.4^{a}	26.0^{ab}	22.5^{b}	4.67	0.014
Octanal	21.9^{a}	16.0^{b}	20.4^{ab}	1.62	0.046
Nonanal	15.9^{a}	12.3^{b}	13.7^{ab}	0.89	0.028
Decanal	4.51^{b}	4.34^{b}	4.99^{a}	0.09	<0.001
Methyl Butyrate	5.38^{a}	4.98^{b}	4.98^{b}	0.05	<0.001
Methyl Heptanoate	4.77^{a}	4.75^{b}	4.76^{b}	0.004	<0.001
Methyl Octanoate	4.19^{a}	4.17^{ab}	4.15^{b}	0.01	0.034
Nonanoic Acid	5.89^{b}	5.92^{b}	6.06^{a}	0.04	0.009
Octane	8.45^{b}	10.25^{ab}	10.63^{a}	0.61	0.042
Decane	2.90^{b}	3.07^{b}	3.38^{a}	0.05	<0.001

[1]Standard error of the mean (SEM) pooled among all mean comparisons.
^{a,b}LS means within a row lacking a common superscript differ ($P < 0.05$).

IV. CONCLUSION

Dried meat exudate added to ground beef influence volatile flavor compound formation. Sensory evaluation should be conducted to confirm impact on beef flavor attributes.

ACKNOWLEDGEMENTS

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SESSION 11

Meat and Health

Thursday 22 August 2024

ADDING AN ANTIOXYDANT COCKTAIL TO PIG FEED REDUCED LUMINAL OXIDATION IN RATS FED A COOKED HAM DIET FROM SUPPLEMENTED REARING

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I. INTRODUCTION

Excessive consumption of cured meats correlates with an increase in the risk of developing colorectal cancer [1]. Different hypotheses have been explored to better understand the substances that are responsible for an increase in risk. Research has identified *N*-nitrosocompounds, such as carcinogenic *N*-nitrosamines [*i.e. N*-nitrosodimethylamine or *N*-nitrosodiethylamine], and the ability of heme iron to catalyse lipid oxidation and nitrosylation with nitrosylated heme iron. Lipid oxidation induces the formation of geno- and cytotoxic terminal aldehydes [malondialdehyde (MDA) and 4-hydroxy-2-nonenal]. To limit cancer risk, some studies have found that adding antioxidants or polyphenols when preparing processed meats inhibited these reactions [2]. Dietary vitamin E supplementation of pig feed may also be of interest to protect cured meat from the formation of nitrosamines and aldehydes in dry sausages [3]. In animal models, vitamin E supplementation can reduce the risk of preneoplasic lesions in rats fed with cooked sausages [4]. In the present study, we assessed the effect of adding a mix with of vitamin E and plant extracts in pig feed on the formation of faecal nitrosylation, nitrosation and peroxidation biomarkers associated with risk of colon cancer in rats fed a diet based on cooked ham from supplemented animals compared to rats fed a diet based on cooked ham from control animals.

II. MATERIALS AND METHODS

A batch of 54 pigs, from Piétrain sires, were divided into three groups: a group fed a basal diet and two supplemented groups, fed a basal diet supplemented at 2g/kg (Ax1: 100 ppm of vitamin E + polyphenol with 8.6 ppm gallic acid equivalent [5]) or at 4g/kg with the same supplementation (Ax2) during the finishing period. The pH1 (45 min *post-mortem (pm)*) and ultimate pH (pH24 ext., at 24h pm) were performed on *Semimembranosus* and *Long head triceps brachii* (LHT). Meat colour (colorimeter Konica Minolta, Japan) and drip loss (EZ method) were measured on LHT. Three batches of cooked ham models (2.5 kg/batch) were produced with pork shoulder from the standard group (DCNO: Dark Cooked with Nitrite and Oxidized) or from antioxidant-supplemented groups (DCNOAx1 and DCNOAx2). A single brine was made with salt (17g/kg), sodium nitrite (102 ppm), sodium erythorbate (500 ppm), and dextrose (5g/kg). After mixing and tumbling for one night, pieces of meat were cooked to an internal temperature of 68.5°C for 48 min and stored in vacuum packaging for 14 days at 4°C under UV lamp. At day 0, cooked hams were analysed for: vitamin E (NF 12822), fat, protein, moisture (NF V04-403 - NF V04-407 - NF V04-401) and, total and nitrosylated heme iron (Hornsey). Lipid oxidation was assessed by the TBARS method (Thiobarbituric Acid Reactive Substances) at day 0 and day 14. Twenty-four rats (Fischer 344) were assigned to 4 groups and received experimental diets for 15 days: *1)* diet without cooked ham (CON), *2)* diet with DCNO ham, *3)* diet with DCNOAx1 ham, and *4)* diet with DCNOAx2 ham. Faeces were collected for 24h and stored at -20°C until analyses: haem, luminal aldehydes (TBARS), cytotoxicity, ATNC (Apparent Total Nitroso Compounds).

III. RESULTS AND DISCUSSION

Supplementation had no effect on pH, lightness (L*) and drip loss. The vitamin E content increased respectively by a factor of 2.57 and 3.03 in DCNOAx1 and DCNOAx2 (Table 1). The predominant form was α -tocopherol. The moisture, fat and protein amounts were similar as those of a cooked, derinded and defatted ham. Total heme iron was higher, as desired due to the use shoulder. Supplementation had no impact on nitrosylation. Lipid oxidation was reduced in DCNOAx2 ($p < 0.03$).

Table 1. Chemical composition of cooked ham models.

	Vit. E (mg/100g)	Moisture (%)	Fat (%)	Protein (%)	Heme (mg/kg)	Heme-NO (mg/kg)	TBARS (mg/kg) D0	TBARS (mg/kg) D14
DCNO	0.225	74.43	3.72	19.02	111±4.9a	70±4.6	0.309±0.03	0.266±0.03
DCNOAx1	0.579	74.09	4.04	19.24	94±5.1b	69±2.6	0.314±0.05	0.295±0.01
DCNOAx2	0.683	74.60	3.33	19.16	104±2.1ab	73±1.2	0.269±0.02	0.247±0.03

Heme: Total heminic pigment; Heme-NO: nitrosylated heme iron; Means values ± SE (n=4); Values with different letters (a–i) are significantly different ($p< 0.05$).

The DCNO diet induced a significant increase of three endogenous biomarkers: ATNC, Heme-NO and TBARS (Table 2). The antioxidant-enriched rearing in DCNO-Ax1 and DCNO-Ax2 diets had no effect on ATNC and Heme-NO. Interestingly, the DCNOAx2 diet significantly reduced the formation of luminal aldehydes (MDA). This effect significantly reduced cytotoxic and genotoxic activities of faecal biomarkers in DCNO-Ax1 and DCNO-Ax2 groups (↗ cellular viability) compared to the DNCO group.

Table 2. Effect of dietary supplementation during pig rearing on fecal biomarkers modulation in rats.

Diets	Rat Numbers	Nitroso-compounds ATNC (µmol/L)	Nitroso-compounds Heme-NO (µmol/L)	TBARS µmol/L Eq. MDA	Cellular viability (%)
CON	6	4.06±3.81	4.71±2.93	13±0.9	47.2±4
DCNO	6	18.47±4.25*	11.35±3.01*	32.8±5.2**	19.8±6.5**
DCNOAx1	6	18.50±5.78*	12.71±3.06*	29±8.6**	36.5±5.6#
DCNOAx2	6	19.00±5.45*	17.67±3.46*	25.5±1.4#	35.7±7.4#

Data are mean ± SEM (n=10); * significantly different of CON ("*" p <0.05, "**" p <0.01); # significantly different of DCNO (p value <0.05).

IV. CONCLUSION

This study shows that the enrichment in α-tocopherol and plant extracts during pork rearing decreases the formation of faecal aldehydes in rats fed a diet based on cooked hams from supplemented pigs. This protective effect was observed on cellular viability increasing when meat was enriched with an antioxidant during animal rearing. It should be investigated (i) whether these endogenous changes are associated with a protective effect against the promotion of colorectal carcinogenesis and (ii) whether effects on the formation of luminal TBARS are also observed in the context of the reduction or removal of nitrites recently associated with high luminal peroxidation [6].

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SALT REDUCTION STRATEGIES FOR MEAT PRODUCTS

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I. INTRODUCTION

The excessive consumption of sodium contributes to numerous health issues, particularly cardiovascular diseases. The World Health Organization (WHO) [1] set a goal of reducing salt intake by 30% by 2025, driving a growing demand for food items with lower sodium content. However, sodium chloride ensures the safety and the development of desirable sensory characteristics of meat products [2]. Potassium chloride [3] and oleoresins of aromatic herbs [4] are potential alternatives to reduce sodium chloride and warrant further investigation. The present study aims to develop and formulate a low sodium cured meat sausage (CMS) using KCl and oleoresins as sodium replacers, and to assess its quality attributes during shelf life.

II. MATERIALS AND METHODS

Three batches of each CMS were formulated and produced in three different working days: C1-Control1.5% NaCl; F1- 1% NaCl and 0.5% KCl; F2- 1% NaCl and 0.5% encapsulated oleoresins with 0.3% KCl; F3- 1% NaCl and 0.5% encapsulated oleoresins without KCl. The mixture rested for 48h at 7ºC, then it was stuffed into a natural thin pork casing and tied in a horseshoe shape. Sausages were dried and cold smoked for 6h at 25ºC, with smoke generated by burning holm wood scraps. After this first cycle of drying/smoking, sausages were kept a 4ºC for 12h. The smoking and maturation (curing) steps were repeated in three consecutive days. After the third smoking cycle, sausages remained at 4ºC for five days. Sausages were then vacuum packed and stored at 4ºC. Analysis were performed on the meat seasoning phase, before stuffing, on the final product, and during storage (2, 4, 6 months). Microbial analysis was performed according to ISO Standards: Lactic Acid Bacteria (LAB), Coagulase Negative Staphylococci (CNS) *Enterobacteriaceae* counts, and *Listeria monocytogenes* detection and counts. Water activity (A_w), pH, and TBARS were evaluated. The L*a*b* color was measured with a Konica Minolta CR-400/410 (Konica Minolta, Japan) illuminant D65. Sensory analysis was carried out on the final products. A consumer test was performed with 97 participants, which included a hedonic evaluation, a yes/no question for the presence of metallic taste, and Just About Right (JAR) scale to assess the adequacy of saltiness.

III. RESULTS AND DISCUSSION

The different CMS formulas for salt reduction presented no difference regarding A_w and pH. The sausages' color was significantly ($p<0.0001$) affected by the formulations, presenting F2 sausages the higher a* (15.79). The effect of formulations was highly significant ($p<0.0001$) on TBARS, with a sausages prepared with KCl and, or oleoresins (F1= 0.59 mg MDA/kg, F2= 0.45 mg MDA/kg and F3= 0.39 mg MDA/kg) presenting lower lipid oxidation than the control (0.77 mg MDA/kg). The antioxidant

effect of aromatic plant oleoresins to meat product formulations could be explained by bioactive compounds, such as phenolic compounds [5]. The absence of *L. monocytogenes* in 25g of the final product was confirmed. These pathogens counts were always below 10 cfu/g till the end of storage (6 months). The different formulas had no effect on *Enterobacteriaceae*, LAB, or CNS counts. LAB and CNS populations presented an increase only during the period of manufacture but not during the shelf life. A metallic taste is often associated with salt substitutes containing potassium chloride. Despite this association, the chi-square test showed no significant association ($p>0.05$) between the presence of a metallic taste and the inclusion of KCl in the formulations, even for the highest concentrations tested (0.5% KCl). When evaluating consumer' adequacy evaluation of saltiness using the JAR test, it is commonly agreed upon that if approximately 50% of consumers consider the characteristic to be "just about right," the product is ready to launch in the market [6]. Hence, in both CMS formulation control (55%) and F1 (54%), the salt content was evaluated as ideal by more than 50% of consumers. In CMS formulas with encapsulated oleoresins, the salt content was considered ideal by 42% (F2) and 45% (F3) of the consumers. These results suggest that encapsulated oleoresins in CMS have potential, but still needs further optimization. Non-ideal evaluations, namely low salt content, were evaluated by 31% (C), 37% (F2), 43% (F2), and 44% (F3) of consumers, which resulted in penalties of 1.58, 0.77, 1.30, and 1.25 units in the 9-point hedonic evaluation, respectively. Nevertheless, hedonic evaluation results showed that all samples were classified above the center of the scale (5 in the 9-point scale), with no statistical differences ($p>0.05$) found between formulations.

IV. CONCLUSION

The salt content of CMS produced with 1% NaCl and 0.5% KCl was considered ideal by enough consumers to launch the product in the market. Results from this study show that it is possible to achieve 33% sodium replacement with KCl without jeopardize CMS organoleptic characteristics nor its safety. The KCl and plant oleoresins used in CMS delayed lipid oxidation during storage. All CMS produced with KCl (F1 and F2) improved the desirable red color. Further studies are required to optimize the use of encapsulated oleoresins, to achieve ideal saltiness. The formulation with 1% NaCl and 0.5% KCl might be a solution to reduce sodium intake associated with the consumption of meat products.

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SESSION 13

Consumer Topics

Friday 23 August 2024

MICROBIOLOGICAL AND SENSORY ACCEPTABILITY OF HAM: EFFECT OF HIGH PRESSURE AND BIOPRESERVATION

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I. INTRODUCTION

In the meat industry, current health recommendations aim to reduce sodium nitrite levels in cured meats. In this context, we studied cooked ham stabilized by combining high pressure and biopreservation with lactic acid bacteria to reduce nitrite levels to 25 ppm (compared with 150 ppm max allowed by European regulation in this product). The shelf life of meat products is generally defined by the count of endogenous spoilage bacteria, and by the development of unacceptable odors and/or appearance. Thus, microbiological and sensory criteria are intimately linked, and mainly contribute to products acceptability by the consumer. In this study, sensory characteristics and microbial counts were monitored during refrigerated storage of treated and control diced cooked ham.

II. MATERIALS AND METHODS

Cooked ham was “home”- manufactured according to Rakotondramavo et al. (2019) [1]. *Longissimus dorsi* was used as testing ham-type muscles. The cooked hams were cut into 1.5 cm cubes. Control samples were vacuum-packed (80 mbar) and stored 10 days at 4 °C followed by 20 days at 8 °C (accelerated aging). Treated samples (biopreserved and pressurized BLc+HP) were first inoculated by spraying a suspension (10^6 CFU/g of ham) of the protective strain *Lactococcus lactis* CH-HP15 [2], then placed at 4 °C for 1 h, and transferred to PA/PE bags and vacuum-packed for high-pressure processing (500 MPa, 5 min, 20 °C) [1]. Treated samples were then stored in the same conditions as control samples. Those conditions (10 days at 4 °C followed by 20 days at 8 °C, Figure 1.C) were chosen in order to promote alteration bacteria growth (if present). *Lactococcus lactis* CH-HP15 was selected among a collection of 63 protective strains for its antagonistic activity against spore-forming bacteria and its ability to resist and regrow following HPP [3].

Enumerations were realized on PCA (total aerobic mesophilic counts) and M17 (lactococci counts) plates. After enumeration, 15 to 20 colonies were picked from PCA plates for identification (Isolation on FTA® membrane, 16S rDNA PCR amplification then sequencing).

A sensory evaluation was carried out to assess the odor (triangular test, 30 untrained panelists), taste and global appreciation (hedonic test, 60 untrained panelists) of ham (control and BLc+HP).

III. RESULTS AND DISCUSSION

Firstly, the bacterial enumeration after accelerated aging showed a progressive increase in mesophilic aerobic counts, from 4 log CFU/g at day 1 (D+1) to 8 log CFU/g at D+30, that was similar in the control and treated samples (Figure 1.A). Moreover, enumeration on M17 plates (Figure 1.B) shows that most aerobic bacteria was composed of lactic bacteria.

To assess the efficiency of the combined treatment, species identification was carried out on the biopreserved and pressurized samples at D+1, D+15 and D+30, stored with a break in the cold chain. The results of the identification showed that at D+1 and D+15, 100 % of the bacteria isolated

corresponded to *L. lactis*, the bioprotective strain. However,at D+30, we observed the growth of another species of lactic acid bacterium, *Carnobacterium sp*. (33 %).

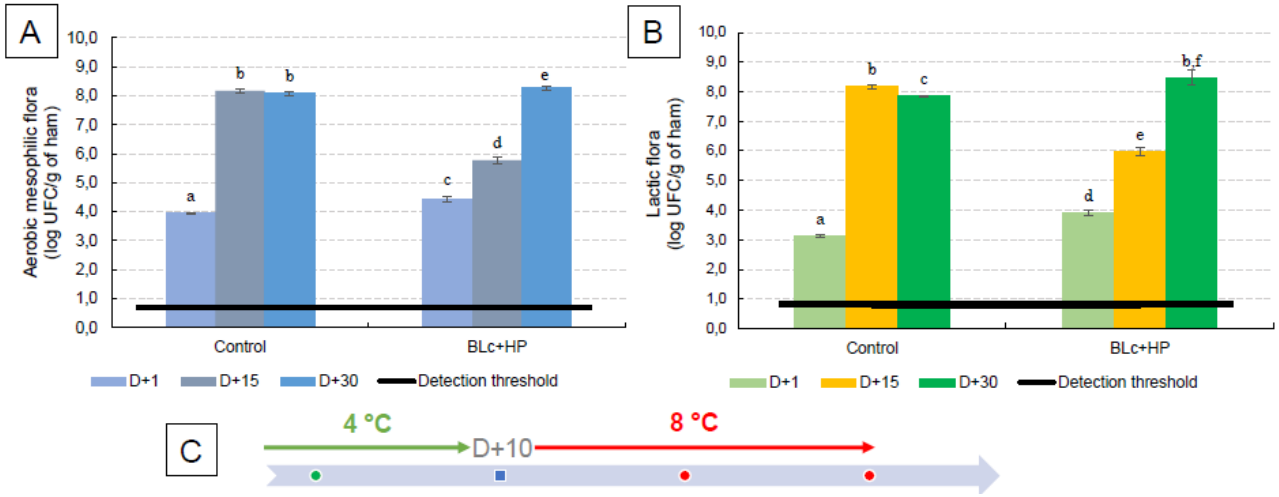

Figure 1. Evolution of total aerobic mesophilic bacteria (A) and lactic acid bacteria (B) during accelerated aging (C).

Regarding olfactory sensory analysis, the judges noted no significant difference in odour between the samples at D+1 but perceived significant differences at D+15. A hedonic analysis showed no difference in colour between the control or treated samples. However, the panel showed a clear preference regarding the overall appreciation and the taste of the treated product. The panelists noted the development of milky and/or fruity aromas.

IV. CONCLUSION

The use of *L. lactis* CH-HP15 combined with high-pressure treatment would be an interesting process for maintaining the microbiological quality of nitrite-reduced cooked ham. After high-pressure treatment, *L. lactis* could grow and dominate the endogenous microbiota during storage, inhibiting spoilage bacteria and pathogens, although other bacterial species could be observed at the end of the shelf-life, as previously observed [2]. However, modification of odours probably linked to the metabolic activity of *L. lactis* was also observed as storage progressed. It would be interesting to identify the volatile compounds formed.

Further studies remain to be carried out in order to promote the recovery of the biopreservation bacterium and to verify on different products the possibility of extending the shelf-life of meat products even in the absence of nitrites or other preservatives.

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SENSORY TRAITS IMPORTANCE DRIVEN BY THE BEEF CONSUMERS: PATHWAYS TO A 3G GLOBAL BEEF EATING QUALITY PREDICTIVE SYSTEM TO MEET CONSUMERS' EXPECTATIONS

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I. INTRODUCTION

The meat industry faces several challenges and predicting eating quality is one of them. Several commercial grading systems are used globally to trade beef, but only one is scientifically based on consumer expectations, the MSA grading system developed in Australia. In Europe, the actual commercial grading system for carcass conformation and fat distribution EUROP doesn't align with consumer's meal experiences. This was proven over the past 15 years with meat eating quality research trials using untrained consumers across Europe [1]. Since 2017 the collaborative platform hosted by the International Meat Research 3G Foundation (IMR3GF) has compiled the consumer sensory data collected using UNECE Beef Eating Quality protocols. The IMR3GF was able to design a European predictive model based on consumer sensory responses across many countries and over 25,000 European consumers.

II. MATERIALS AND METHODS

The approach of using common standards for meat products and sensory evaluation ensures data compatibility and enables reliable consumer sensory estimates to be developed [2]. During the sensory evaluation, each consumer is served 7 samples and each sample is tested by a total of 10 consumers. The data used for this approach represents consumer answers conducted in total on 11 muscles over the last decades in European countries (Poland, France, England, Northern Ireland, Wales, Ireland). Each consumer rated the 4 different variables, tenderness, juiciness, flavour and overall liking on a 100-line scale after eating each sample, 0 representing dislike and 100 like for each variable. The ethical standards were accepted to conduct the sensory sessions with untrained consumers. The potential hypothesis is whether a cultural effect would be observed amongst the consumers from different countries regarding beef preference.

III. RESULTS AND DISCUSSION

The cooking method grill is used in this study under the same conditions and doneness preference as medium rare in all countries except France with rare doneness. The scores given by the consumers for each variable (tenderness, juiciness, flavour, overall liking) were analysed and the weightings for each variable are represented Table 1.

Table 1 – Weightings by country

ConsCountry	Count	Tenderness	Juiciness	Flavour	Overall Liking
FRANCE	10,478	0.331	0.10	0.332	0.327
IRELAND	4,969	0.259	0.096	0.378	0.268
N. IRELAND	34,722	0.25	0.083	0.342	0.325
POLAND	42,397	0.237	0.057	0.397	0.308
WAL & ENG	10,907	0.312	0.104	0.288	0.295
N=	103,473	0.278	0.088	0.347	0.305

The weightings for each variable were determined including the 4 variables (SQ4). We observed a similar trend within the countries with consumer giving less importance to juiciness and more to beef flavour and tenderness.

As observed Table 1, the variable weightings slightly differ by countries, but the average remains the same. The equation used to deliver an accurate eating quality predictive score is 0.3tn+0.1ju+0.3fl+0.3ov for all the European consumers.

IV. CONCLUSION

The cultural differences were not driving the weightings on these experiments with untrained beef consumers. However, the flavour and tenderness variables were stronger and currently sought by beef consumers. These data have enabled the Foundation to develop a European predictive model based on research trials. The database contains data connecting cattle, carcase treatments, cuts and cooking styles to consumer answers on eating quality. The predictive model is an evolutive tool with further eating quality accuracy and scope developed with greater data. As all industry revenue directly relates to the consumers judgement of value, with the most critical component meal satisfaction, industry profitability can be enhanced by delivering consistent eating quality through strong commercial brands built on a solid scientific foundation.

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Short Papers-POSTER

SESSION 2

Sustainability

Monday 19 August 2024

EFFECT OF DIETARY SUPPLEMENTATION WITH AGRO-INDUSTRIAL BY-PRODUCTS AND FLAX SEED ON THE PHYSICOCHEMICAL QUALITY OF ORGANIC FREE-RANGE ROOSTER MEAT

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I. INTRODUCTION

The growing consumer demand for changes in animal welfare has led to the promotion of extensive rather than intensive agricultural systems. In this sense, better animal health can be favored, and also the obtaining of more sustainable and better-quality meat [1]. On the other hand, the use of non-conventional feeds such as agro-industrial by-products contributes positively to environmental, social, and economic sustainability, and also to a potential promotion of the health and well-being of poultry [2]. It is worth mentioning that this type of supplementation might affect some quality parameters of the meat, such as color, texture, or water loss during cooking, influencing consumer acceptance [3]. Therefore, the aim of this work was the evaluation of different physicochemical parameters in the meat of free-range roosters fed with several agro-industrial by-products, such as beer bagasse (BB) and olive pomace (OP), and flax seed (FS), an underused raw material in animal feeding in Spain.

II. MATERIALS AND METHODS

II.I. Animals and feeding

This study was carried out with male roosters raised for 3 months. Then, they underwent a fattening phase in semi-freedom pens for another 4 months until sacrifice. For this last stage, the birds were divided into 4 batches: control (CO), BB, OP, and FS. In the CO batch, the roosters were fed exclusively with corn, wheat, and peas. This mixture was added with 5% (w/w) of BB, OP, and FS in each of the other batches, as appropriate.

II.II Sampling

Each batch consisted of a total of 40 roosters. After the first 24 hours of slaughter, breasts from 10 randomly sampled carcasses were used for physicochemical determinations of pH, color, represented by the CIELAB space (L^*, a^*, b^*), cooking loss, and texture, using the Warner-Bratzler test, which was represented by the shear force, firmness, and the shear energy to cut the piece of meat. Both the cooking loss and texture analysis were performed according the procedure used by Aurora et al. [4].

II.III Statistical analysis

One-way analysis of variance (ANOVA) was performed for all the variables evaluated using the IBM SPSS Statistics 23.0 program (IBM Corporation, Somers, NY, USA). Least square means were separated using Duncan's *post hoc* test (significance level $P<0.05$).

III. RESULTS AND DISCUSSION

After the next 24 h *post-mortem*, the breast meat of all batches showed normal pH values (Table 1). Except for the OP batch, the meat from the experimental batches exhibited significantly different values ($P<0.05$) compared to the CO batch. Regarding color, no differences were observed in the lightness and redness of the samples. However, yellowness showed significantly lower values ($P<0.05$) in the OP and FS batches than in the CO batch. Usual cooking loss values were obtained in all meat samples tested. The maximum value was reached in the CO batch (14.77±1.11%), while the minimum was in the OP batch (10.77±1.58%). The ability of meat tissues to retain water is related to tenderness and juiciness [5], and generally determines the quality of fresh meat.

The texture of the breasts showed significant differences (P<0.05) among the experimental groups (Table 1). Thus, the batch of roosters fed with BB had a lower shear force than the CO, while the batch fed with OP presented a higher work. Recent studies also found an effect on meat texture when varying the finishing diet in poultry [5,6].

Table 1 – Effect of supplementation with different agro-industrial by-products and flax seed on the pH and color of organic free-range rooster breasts (n = 10).

	Batch				SEM
	CO	BB	OP	FS	
pH	5.89^{a}	5.63^{b}	5.81^{a,c}	5.72^{b,c}	0.22
*L**	51.07	52.42	51.22	49.92	0.44
*a**	1.03	0.62	0.58	0.78	0.15
*b**	11.9^{a}	11.68^{a,b}	10.72^{c}	10.83^{b,c}	0.17
Cooking loss (%)	14.73^{a}	13.56^{a,b}	10.77^{c}	13.31^{b}	0.31
Shear force (N/cm^2)	1.68^{a}	1.4^{b}	1.65^{a}	1.56^{a,b}	0.39
Firmness (N/s)	0.53	0.46	0.52	0.53	0.14
Shear energy (N·mm)	5.05^{a,b}	4.36^{a}	5.62^{b}	4.68^{a,b}	0.20

CO: control; BB: beer bagasse; OP: olive pomace; FS: flax seed; SEM: standard error of mean; $^{a-c}$Means in the same row not followed by a common superscript letter are significantly different (P<0.05; Duncan's test).

IV. CONCLUSION

The inclusion of food by-products (BB and OP) and FS in the finishing diets of organic free-range roosters had special influence in parameters, such as cooking loss and texture, while color was hardly affected. These outcomes could be correlated with certain sensory attributes, so further research should be focused on this direction.

ACKNOWLEDGEMENTS

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Bulls technological meat quality influenced by breed and rearing intensity

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I. INTRODUCTION

Production systems of bulls for meat production are often quite intensive and focuses on carcass composition and hence the production economy relates to rearing efficiency (feed efficiency, growth, slaughter age etc.) and carcass payment according to the EUROP classification system. Insemination of dairy cows using beef breed semen allows producers to add value of offsprings by increasing carcass yields and overall value. This adaption has great potential in Sweden, where approximately 85% of all dairy cows are still bred with dairy semen [1]. However, according to previous research, best paid carcasses according to the EUROP system does not automatically result in meat with the best eating quality [2]. Therefore, it is important to evaluate how such production systems would affect eating quality of the meat. Even if eating quality is a subjective measure and therefore differ depending on personal preferences, there are however some quality measures that can be highlighted as more important from a consumer point of view, such as colour, tenderness and juiciness. The aim of this study was therefore to investigate how purebred dairy bulls and dairy-beef crossbred bulls raised in either a low-intensity production system (slaughter age 18 months) or a high-intensity production system (slaughter age 15 months) would affect the technological parameters of meat quality; color (L*, a* and b*), thaw loss, cooking loss and Warner-Bratzler Shear Force (WBSF).

II. MATERIALS AND METHODS

This study compared meat quality attributes in meat from 69 bulls reared indoors. The study included 35 bulls of dairy breed (15 Swedish Red and 20 Swedish Holstein) and 34 crossbred bulls (15 Swedish Red × Angus and 19 Swedish Holstein × Angus). The bulls were fed either a high-intensity (64% concentrate) or a low-intensity (44% concentrate) diet *ad libitum* and were slaughtered at 15 or 18 months of age. The feed used was grass-clover silage. After slaughter, all *M. thoracis et lumborum* from the right side were aged at 4°C for seven days before being frozen at -18°C until analysis. All meat samples were weighed to get both thawing loss and cooking loss. All thawed meat samples were tested for colour (L*, a* and b*) and WBSF was measured on cooked meat samples. Data was analyzed using Proc Mixed in SAS with pen as random effect (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). The study was ethically approved by the Ethics Committee on Animal Experiments in Gothenburg (case number 187-2014).

III. RESULTS AND DISCUSSION

Thawing loss, cooking loss, meat colour and WBSF are all presented in table 1. No significant interactions between breed and feed intensity were found. Differences were found for all colour parameters and thawing loss when comparing breeds. The beef-crosses had higher L*, a* and b* values compared to the pure dairy bulls. Previous research has suggested that trained panelists were able to detect a difference of 0.95 in a* and 0.9 in b* [3]. In this study greater differences for lightness, redness and yellowness could possible let consumers perceive a visual difference. The detectable difference may however not necessarily lead to consumer refusal in a purchase situation. Thawing loss differences indicate a higher loss in beef-crosses and in the high intensity group. Chambaz*, et al.* [4] also found differences in fluid losses comparing different beef breeds. Hence, an

explanation for increased thawing loss in the present study could be differences in muscle composition due to the breed or age at slaughter as a result of the different rearing intensities. As the numerical differences were small; 0.8% points for breed and 1.0% points for intensity, this might therefore not have any large impact on the eating quality. There were no effect on tenderness (WBSF), however, the numeric values of WBSF are still important to discuss, and Huffman *et al.* [5] stated an upper limit of 40.2N for consumer satisfaction and Miller *et al.* [6] scribed meat of 45.1N to be slightly tough. This suggests that meat in the current study has WBSF values close to values of beef considered tender.

Table 1 – Colour parameters, fluid losses and shear force values (WBSF).

Traits	Breed		Intensity		SEM[1]	P-value[2]	
	Beef-cross	Dairy	High	Low		Breed	Intensity
n	34	35	36	33			
L* (Lightness)	37.2[a]	32.5[b]	35.4	34.3	1.21	0.0265	0.5281
a* (Redness)	23.1[a]	20.9[b]	21.8	22.1	0.42	0.0068	0.6037
b* (Yellowness)	11.3[a]	9.2[b]	10.2	10.2	0.25	0.0004	0.9834
Thawing loss (%)	5.3[a]	4.5[b]	5.4[a]	4.4[b]	0.19	0.0157	0.0055
Cooking loss (%)	25.0	25.8	25.5	25.3	0.44	0.2814	0.7301
WBSF (N/cm$^{2)}$	42.0	40.6	41.1	41.4	2.66	0.7323	0.9403

[1] Standard error of the mean. [2] Differences considered significant at P<0.05. a-b Mean values within rows with different superscripts differ significantly (p<0.05).

IV. CONCLUSION

The results from this study show that breed had a larger effect on technological meat quality attributes tested than rearing intensity. In this study, differences for lightness, redness and yellowness may not have a negative impact from a consumer perspective. WBSF values in this study may be close to values of beef considered acceptably tender.

ACKNOWLEDGEMENTS

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Lactate measurement in pig blood at exsanguination as predictor for meat quality

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I. INTRODUCTION

The lactate content in pork is an important factor affecting meat quality [1], particularly in terms of its water holding capacity, tenderness, juiciness, and flavor. Lactate is produced during glycolysis, which is the breakdown of glucose in muscle cells. In living animals, lactate levels are typically low. However, after slaughter, muscle glycogen is converted into lactate, causing a decrease in pH, a process known as postmortem glycolysis. The rate and extent of postmortem glycolysis can impact meat quality attributes, by promoting the breakdown of connective tissue, activates enzymes responsible for tenderization, by affecting the water-holding capacity and affecting biochemical processes that contribute to meat flavor development during cooking. High levels of lactate have negative effects causing pale, soft, exudative meat. To manage lactate levels, factors such as pre-slaughter handling, animal genetics, stress reduction before slaughter, and post-slaughter processing techniques are important considerations for pork producers. In earlier research there has also been relation noted between the lactate content in blood at exsanguination and the final pork quality [1]. A positive relation between preslaughter conditions and blood lactate levels at exsanguination were also noted [2]. This indicates that measuring lactate level at exsanguination can become a novel quantified way to relate the preslaughter factors with the final meat quality.
This study aims to explore the relation between the lactate levels at exsanguination with the final meat quality parameters in a commercial slaughterhouse in the Netherlands.

II. MATERIALS AND METHODS

The study involves data from a trial carried out in May 2023 at a slaughterhouse owned by Vion Food, the Netherlands. A total of 80 pigs were slaughtered, and blood samples were taken immediately after stunning and sticking. These samples were measured with a hand-held lactate analyser (Lactate Scout 4, EFK Diagnostics, United Kingdom). After exsanguination, various meat quality parameters were measured, including drip loss, pH at 45 minutes, 3 hours, 6 hours, and 24 hours, as well as Minolta L, a, b colour values. All measurements were then correlated with the blood lactate values at exsanguination through correlation analysis, and correlation plots were created for visual simplification. Out of the 80 pigs, complete data from all measurements were available for 74, thus the analysis presented is based on 74 samples. All analyses were performed using MATLAB (MathWorks, Natick, MA, USA).

III. RESULTS AND DISCUSSION

Correlations of several meat quality parameters are plotted against the lactate values of blood during exsanguination (Figure 1). The lactate values show a slight positive correlation with drip loss and a negative correlation with pH at 45 minutes, 3 hours, and 6 hours. pH at 24 hours shows almost zero correlation with lactate value. Furthermore, lactate values exhibit a slight positive correlation with Lab colour values. Drip loss shows a positive correlation with the Lab values. pH at 45 minutes has a positive correlation with pH at 3 hours and 6 hours. pH shows almost no correlation with Lab values. Additionally, drip loss, pH at 45 minutes, 3 hours, and 6 hours shows almost zero correlation with pH at 24 hours. An earlier study [1] also reports a

positive correlation between lactate and drip loss and a negative correlation between lactate and pH. Based on such correlations, the earlier study suggested that lactate at exsanguination is predictive of the rate of early post-mortem metabolism.

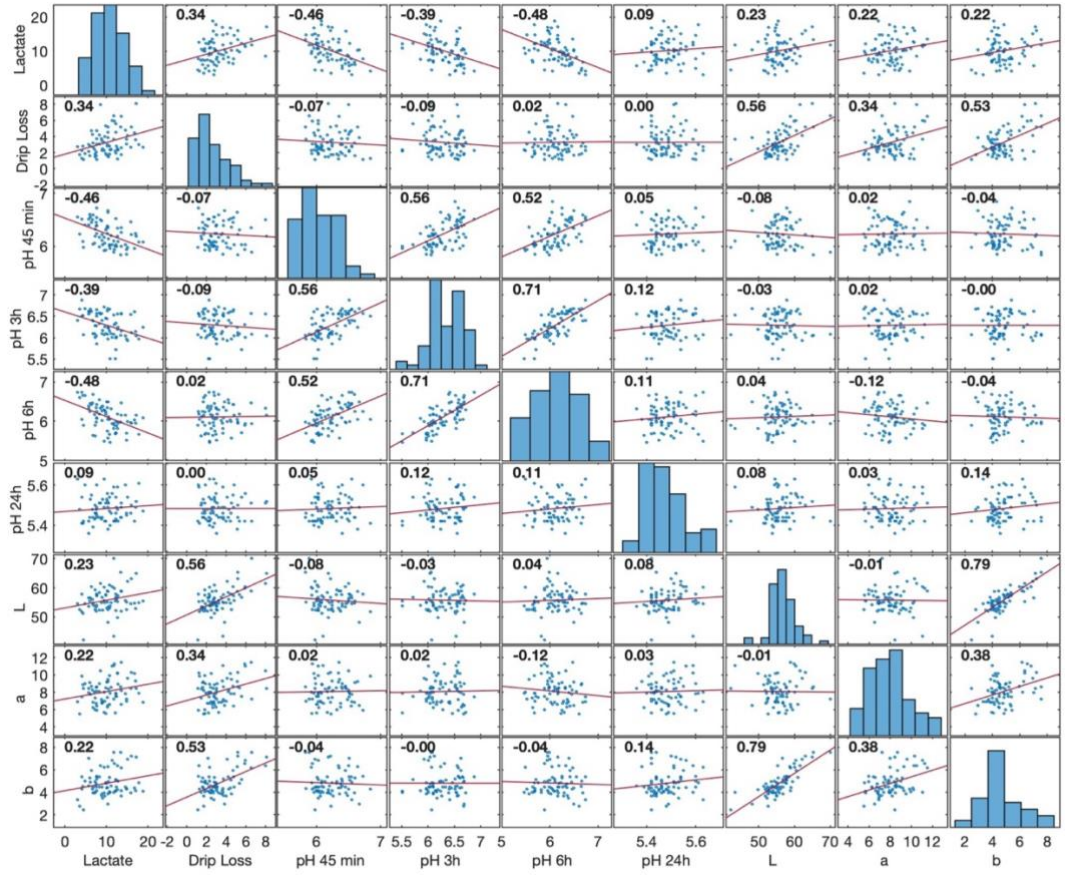

Figure 1: A summary of correlation plots between blood lactate levels at exsanguination and different meat quality parameters.

IV. CONCLUSION

This study explored the correlation of lactate values of blood at exsanguination and several of the meat quality parameters at a commercial slaughterhouse in the Netherlands. The results suggested that like earlier reported results [1], the lactate values at exsanguination carried correlation with meat quality parameters such as drip loss and pH. With drip loss, lactate carried positive correlation while with pH a negative correlation. Some positive correlation with Minolta Lab values was also note for lactate. Overall, the blood lactate values at exsanguination are found to be correlated to meat quality parameters. An in-line blood lactate measurement system during exsanguination can allow tracking the lactate values for individual animal allowing a better track of meat quality as well as animal welfare.

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THE EFFECT OF BOVAER® SUPPLEMENTATION (3-NITROOXYPROPANOL) ON THE EATING QUALITY OF BEEF

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I. INTRODUCTION

The use of Bovaer or "3-nitrooxypropanol" (3-NOP) as a methane (CH_4) inhibitor in ruminant livestock is projected to become common practice in beef cattle feedlots in the coming decade. Ultimately, the meat quality and consumer satisfaction of grain fed beef is of paramount importance and cannot be negatively impacted by feed additives. Metabolites that result from 3-NOP breakdown in the rumen are rapidly metabolised and lead to no effect on the animal besides methane reduction [1]. In studies by Vyas et al. [2] and Vyas et al. [3] it was reported that no effect on animal performance, live weight or carcass phenotype was observed when crossbred steers were supplemented with 3-NOP in a barley-based high-grain diet, which suggests that beef eating quality traits for tenderness, juiciness, flavour and overall liking will be unaffected. Currently no research has investigated the effect of 3-NOP on the eating quality of beef. The objective of this study was to determine the effect of Bovaer 10® on the intrinsic eating quality of beef.

II. MATERIALS AND METHODS

An 84-day feeding trial was conducted with 48 *Bos taurus* milk tooth steers that were randomly assigned to control and Bovaer 10® (100 mg 3-NOP/ kg DM) treatment groups (n = 24 per treatment; 4 pens of 12 animals per pen). The steers were adapted to a grain finisher ration over 28 days and Bovaer 10® supplementation started on day 10. At 24 hours post-mortem, the carcasses were graded and the *M. longissimus lumborum* (striploin) and *M. gluteus medius* (rump) muscles from each animal were collected. The meat was processed into 25mm steaks for consumer testing. Two samples from each cut were aged for 14 and 35 days. The ageing periods were rotated between the anterior and posterior positions of each primal. The samples used for sensory testing followed the protocol presented by Watson et al. [4]. Following processing, the samples were vacuum packed and frozen at -20 °C until required. Samples were defrosted 24h before the sensory session. The samples were grilled on a Silex grill (Type S-161K OV, Arnsberg, Germany) for 5 minutes (top plate 195 °C and bottom plate 210 °C) to reach a desired internal temperature of 67°C or medium doneness. The steaks were assigned to consumers in a 6x6 latin square design so that the order of sample presentation did not impact the sensory scores. Ten untrained consumers assessed each sample for tenderness, juiciness, flavour and overall liking using a 100 point scale line. In total 480 untrained consumers were used to assess all samples. Consumer meat quality score (MQ4) is a weighted index score of tenderness (30%), juiciness (10%), flavour (30%) and overall liking (30%). The MQ4 score was analysed using linear mixed effect models in R [5] with treatment, cut and ageing period as fixed effects and animal within pen as the random term.

III. RESULTS AND DISCUSSION

There was no effect of 3-NOP supplementation on live animal performance or carcass phenotypes ($P>0.05$). The interaction between treatment (Control or Bovaer 10®), cut (striploin or rump) and days aged (14- and 35- days) was not significant ($P>0.05$) for MQ4 score. There was no effect of Bovaer 10® supplementation on MQ4 score in the striploin (*m. Longissimus lumborum*) or rump (*m. Gluteus medius*) at 14 or 35 days ageing (Figure 1). The MQ4 was significantly affected by days aged ($P= 0.039$). The MQ4 score was 4.25 points lower at 14 days ageing compared to 35 days ageing for the

striploin and rump (Figure 1). MQ4 score was also significantly affected by cut (P=0.014) with the rump being 6.04 points lower than striploin (Figure 1) at both 14 and 35 days ageing.

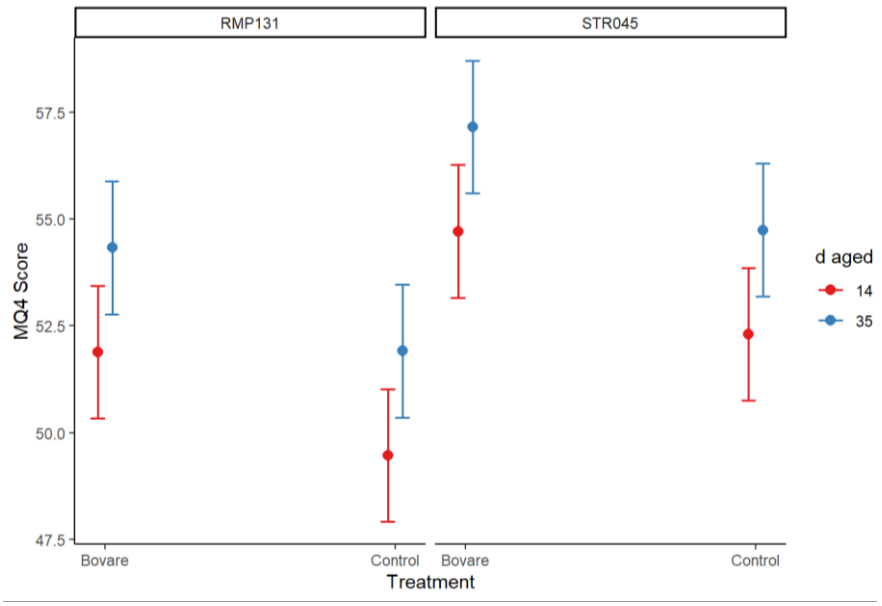

Figure 1. The estimated marginal means with 95% confidence intervals for eating quality score (MQ4) for striploins (STR045) and rump (RMP131) from animals not supplemented (Control) and supplemented with Bovaer 10® (100mg 3-NOP)/ kg DM) at two different post-mortem ageing periods (14 and 35 days)

IV. CONCLUSION

In this study the inclusion of Bovaer 10® (100mg 3-NOP/ kg DM) into high-grain feedlot rations for beef steers had no impact on the intrinsic eating quality of beef for the striploin and rump muscles after 14 and 35 days ageing. A reduced carbon footprint beef product has the potential to lead to greater consumer satisfaction if marketed effectively as a credence quality attribute of beef, however the size of impact of extrinsic marketing on the neurograstronomy of consumers could be beneficial and needs further investigation.

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SUSTAINABILITY OF PRODUCERS OF MEATS AND MEAT PRODUCTS IN SHORT CIRCUITS

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I. INTRODUCTION

Buying trends show that for several years, consumers have increasingly turned to local circuits for their food. This aligns with the food behavior trends identified by Kantar Worldpanel in 2017 : consuming less but better, favoring products with a healthier image. The development of activities by farmers engaging in direct sales allows them to diversify their sources of income and directly benefit from the added value of the products (Le Caro et al., 2007). Engaging in direct sales opens up new social networks and offers a new form of recognition and meaning to agricultural professions (Tabet, 2009; Le Bahers and Paturel, 2013). A report indicates that selling local products stabilizes the income of involved producers, although not necessarily improving it (Dédinger et al., 2021). However, there is a lack of economic data available for use. Another gap is the absence of tools and benchmarks for managing various aspects of the quality of meats and charcuteries adapted to the context of farm production. Mastering the various aspects of quality of these products is essential to meet consumer expectations. Even though the French are very attached to local products from their regions, which have a very positive image, it is necessary to ensure in short circuits a level of quality control that meets consumer expectations. Therefore, the VICTOR project proposes to develop support tools for beef and pork farmers in short circuits to improve their knowledge and control of the various aspects of the quality of their meats and charcuteries.

II. MATERIALS AND METHODS

The study was conducted in four major French areas: Pays-de-la-Loire, Grand Est, Bourgogne, and Auvergne-Rhône-Alpes. These regions were chosen due to their strong presence and growth in short circuits in recent years, as well as their regional geographic specificities. They offer a range of contrasting situations in terms of sales seasonality and product marketing modes. The selected farms had to meet several criteria: they needed to have beef and/or pork herds and practice short circuit sales. Additionally, they needed to have their own cutting and/or processing workshop. The original protocol was to investigate 40 farms, but due to these expectations, the farmers willingness and availability, and economic data accessibility, this study design was reduced to 24 farms. They were surveyed three times: the first survey was sociological, the second focused on the technological part of meat processing, and the third on the economic profitability of the workshop and the time spent working. The computer tool used in this study was specifically created for this mission by combining the expertise of advisers and researchers. This software was designed to directly capture, process, and enhance the technical-economic and working time data collected during the surveys. It includes the section related to the economic profitability of the short circuit workshop and work organization. The economic data used in this study come from the farms' expense and income accounts, as well as depreciation. Economic profitability is calculated using variable and fixed costs. The time spent by farmers and employees on a typical week or weeks was collected using a specific protocol. Each typical week was broken down day by day. This protocol allows for precise accounting of the time dedicated to the short circuit workshop. To conduct the hygiene and technological surveys, an audit grid was developed. This grid includes 10 sections covering aspects such as breeding, product characterization, subcontracting, slaughtering, the workshop, raw materials, knowledge, good hygiene practices, and elements to observe. Each section contains open questions, closed questions, and ratings to assess various aspects of the farmer's practices. The investigators used tablet software to enter responses and take photos during visits to the transformation workshops. The data collected during these surveys were compiled in an Excel

document for further analysis. These surveys provide valuable information to better understand the practices and needs of farmers involved in short circuit sales as part of the VICTOR project.

III. RESULTS AND DISCUSSION

The sample of farms surveyed is described in table No. 1; these are farms producing pork, beef, or mixed livestock. On average, there are 4 marketing channels (farm sales, markets, producers' stores, home delivery, depot-sales). The detailed analysis of economic results revealed significant trends. Pork farms surveyed generally show positive profitability, while beef farms face more pronounced economic challenges. Farms with pigs and beef cattle present more complex average profitability, emphasizing the importance of labor costs. To meet changing demand, product ranges have become much more diversified. This has a direct impact on working time: 7.7 min on average to cut and process 1 kg carcass in workshops with wide ranges; 3.9 min/kg carcass in those with restricted ranges economic challenge met or close to being met in ¾ of the farms surveyed, at the price of a week often full of work for the farmers.

Table 1 - Comparative Overview of Pig and Beef Cattle Farms

	Pigs farms	Pigs&beef cattle farms	Beef cattle farms
Processed volume (tons in carc. weight per year)	15 to 60	15 to 60	10 to 50
Range of products marketed	Extended range	Very wide range	Narrow range
Median cutting & processing time (min/kilo of carc.)	7.2	7.7	4.7
Total product from short-line activity (€/kilo of carc.)	9.74	9.55	9.41
Variable and fixe charges (€/kilo of carc.)	4.28	5.19	4.71
Margin without remuneration of the farmer and purchase price of the animal (€/kilo of carc.)	5.62	4.86	4.18

Regarding aspects related to hygiene and the mastery of the technological quality of meats and charcuteries, although many aspects of food processing are mastered by the farmers, there are still areas where improvements are necessary. Farm-based processing workshops subject to health approval must comply with the same regulations and controls as larger meat and charcuterie processing companies.

IV. CONCLUSION

The results of this study provide avenues for the creation of tools and training that could help farmers refine their practices and ensure the quality of their products. These results raise essential questions about ways to improve the economic performance of farms. It becomes imperative to identify specific expenses that can be reduced and to exploit potential levers to strengthen profitability. In summary, this study provided an in-depth and nuanced overview of the challenges faced by farms practicing short circuits. The results and recommendations derived from them offer valuable guidance for future decisions aimed at optimizing the profitability and sustainability of these farms, thus contributing to the ongoing development of the agricultural sector in various regional contexts.

ACKNOWLEDGEMENTS

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Enteric methane emissions expressed by carcass productivity of *Nellore* cattle raised in different pasture systems

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I. INTRODUCTION

In 2023, the population reached the mark of 8 billion people in the world, and projections indicate that this number will increase to 9.7 billion by 2050 [1]. To meet the growing demand for food, it became necessary to intensify existing production systems while also, mitigating the environmental impacts caused by the increased exploitation of natural resources [2]. As a strategy to enhance productivity in pastures, the consortium of pigeon pea with tropical grasses can meet the nutritional requirements of animals, which showed better performance in terms of weight gain when compared to animals kept in extensive and poorly managed breeding systems. This consortium system can mitigate up to 70% of methane (CH_4) emissions when expressed by average daily weight gain compared to treatment with degraded systems [3]. Therefore, the objective of this work was to evaluate hot carcass weight (HCW) and enteric CH_4 emissions of *Nellore* cattle in an intercropped pasture system containing grasses and legumes.

II. MATERIALS AND METHODS

The study was approved and followed the guidelines of the Committee for the Use and Care of Institutional Animals (CEUA) of Embrapa (nº 05/2016) and the College of Veterinary Medicine and Animal Science of the University of São Paulo (nº 6228200521). The experiment was carried out at Embrapa Pecuária Sudeste, São Carlos, SP, Brazil and included 27 *Nellore* steers, with a body weight of 221 ± 7 kg and 15 months old, were randomly distributed into three treatments with three grazing replicates in a completely randomized design, totaling nine grazing units (11.7 ha in total): 1) Degraded pasture of *Urochloa decumbens* Stapf cv. Basilisk (DEG); 2) Recovered pasture established with a mixture of *U. decumbens* cv. Basilisk and *U. brizantha* (Hochst ex A. Rich) Stapf cv. Marandu (REC); and 3) Intercropped pasture, a mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim (MIX). The CH_4 samples was carried out for two years (2021-2022) during the dry (June) and rainy (January) seasons. In the rainy season (October - March), all animals received mineral mixture supplementation, while in the dry season (April - September), animals from REC and DEG received protein-energy supplementation, and MIX, just a mineral mixture. Enteric CH_4 emissions were determined using the sulfur hexafluoride (SF_6) tracer gas technique. At the end of the experiment the animals were fasted for 16 hours, receiving only water ad libitum, and then weighted. In the same day they were transported to a federal inspected slaughterhouse. Immediately after slaughter, the carcasses were weighed to obtain hot carcass weight. The data were subjected to analysis of variance using SAS PROC MIXED and the means were compared by Fisher's test (5%).

III. RESULTS AND DISCUSSION

Table 1 – Carcass production and enteric CH_4 emissions of *Nellore* cattle under different pasture systems.

Variables	Treatment			SEM	Statistical Probabilities (P value)*
	MIX	REC	DEG		
HCW, kg	298.56^{A}	282.22^{AB}	264.50^{B}	7.76	**0.0191**
CH_4, kg/day	0.2596	0.2621	0.2573	0.0008	0.9146
CH_4/HCW, kg/kg	0.6359^{B}	0.6780^{AB}	0.7101^{A}	0.0280	**0.0422**

^{AB} Capital letters differ (P<0.05) treatments by Fisher's test; * Significant at 5%; MIX, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu intercropped with *Cajanus cajan* (L. Millsp.) cv. BRS Mandarim; REC, mixture of *U. decumbens* cv. Basilisk and *U. brizantha* cv. Marandu fertilized with 200 kg of N-urea ha^{-1} $year^{-1}$; DEG, degraded pasture of *Urochloa decumbens* cv. Basilisk; HCW, hot carcass weight; SEM, standard error of the mean.

In Table 1, the HCW of MIX was higher about DEG, and it is also possible to observe that although CH_4 emissions did not show a significant difference between the grazing systems, MIX treatment was able to dilute carcass emissions. At the same time, DEG presented 12% more emissions per unit of product than MIX.

As demonstrated by Furtado et al. [3], pigeon pea has high levels of nutrients, which increases the energy and protein density of the diet consumed by the animals. In addition, the greatest consumption of pigeon pea occurs during the dry season of the year, a period in which *Urochloa* spp. presents reduced digestibility and crude protein (CP) values, compromising rumen fermentation and causing non-supplemented animals to lose weight. Another advantage is the presence of condensed tannins in pigeon pea plants, a factor that helps ensure that CH_4 emissions do not increase with the additional consumption of this forage. Meo-Filho et al., [4] when studying the effects of pasture intensification on Canchim breed steers, they found lower values of CH_4 per carcass than those observed in this experiment, which can be justified by the choice of breed used, given that the Canchim is a breed crossed with taurine (precocious) animals, the time of experiment and slaughter weight were lower, which also reduced experiment emissions. Furthermore, supplementing the animals with the corn silage produced may also have influenced the lower emissions.

IV. CONCLUSION

The pigeon pea proved to be a great alternative for mitigating CH_4, reducing the need for fertilization and the use of food supplements, producing more sustainable meat.

ACKNOWLEDGEMENTS

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FOSTERING SUSTAINABLE PRACTICES IN INDIGENOUS SLOW-GROWING CHICKEN PRODUCTION: DESIGN OF A REDUCED-SOY DIET FORMULATION

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I. INTRODUCTION

The widespread use of soy in animal feed is driven by its high protein content and favorable amino acid profile [1]. However, the expanding cultivation of this leguminous crop is linked to deforestation, soil degradation, wildlife habitat destruction, and loss of natural grasslands [2], prompting global concerns in the animal feed production sector. On the other hand, increasing consumer awareness of animal welfare is driving demand for products from traditional farming practices. Additionally, slow-growing and native breeds like 'Galiña de Mos' are gaining popularity in the meat market [3]. These factors emphasize the urgent need to explore environmentally friendly feed formulations for free-range animals while maintaining high meat quality. Thus, this study aims to evaluate the impact of reduced-soy feed on the chemical composition and physicochemical parameters of the 'Galiña de Mos' breed.

II. MATERIALS AND METHODS

To achieve this goal, 46 'Galiña de Mos' chickens (32 males and 14 females) were randomly divided into two groups of 23 animals each (16 males and 7 females) after being fed a commercial starter feed for 75 days. One of these groups was fed a commercial feed containing corn and soy, while the other group received a custom feed with additional ingredients such as wheat (10%), peas (6%), rapeseed (3%), and flaxseed (1%), reducing soy content. The feeding period lasted 118 days and took place outdoors. Analyses were performed on the right breast of 15 representative samples from each group. Moisture, protein (Kjeldahl N × 6.25), and ash were measured following ISO standards, while fat content was determined using the American Oil Chemists' Society (AOCS) procedure. Meat quality analysis included pH measurement (after 24 h), color evaluation, water holding capacity (WHC) and texture (Warner-Bratzler test), as described by Pateiro et al. [4]. Additionally, the obtained results were evaluated using one-way ANOVA with SPSS package version 23.0 (IBM SPSS, Chicago, IL, USA).

III. RESULTS AND DISCUSSION

Although diet can significantly influence the chemical composition of poultry meat [3], this trial observed that no parameters were significantly ($P > 0.05$) affected by the diet supplied to the 'Galiña de Mos' breed, except for ash content in roosters, which was significantly ($P < 0.01$) higher in the control group (Table 1).

Table 1 – Influence of reduced-soy feed on the chemical composition of breast meat in the native breed 'Galiña de Mos' (values expressed as mean ± standard error).

	Males			Females		
	Control feed	Reduced-soy feed	Sig.	Control feed	Reduced-soy feed	Sig.
Moisture (%)	74.65 ±0.59	74.59 ± 0.50	ns	73.52 ± 0.39	73.95 ± 1.12	ns
Intramuscular fat (%)	0.10 ± 0.12	0.07 ± 0.04	ns	0.30 ± 0.35	0.47 ± 0.61	ns
Protein (%)	23.96 ± 0.71	24.18 ± 0.52	ns	24.8 ± 0.25	24.42 ± 0.59	ns
Ash (%)	1.13 ± 0.03	1.19 ± 0.04	**	1.20 ± 0.01	1.2 ± 0.03	ns

Sig: significance: * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$); ns: no significant difference

This lack of significant effect could be attributed to the formulation of the reduced-soy feed, which was designed to closely match the proximal composition found in commercial feeds. Similarly, the content of essential amino acids for birds, such as lysine and methionine, was adjusted to mirror those of commercial formulations. As a result, bird metabolism appeared unaffected, maintaining consistent muscle composition regardless of the diet provided. Concerning pH (Table 2), the diet significantly affected its value in both sexes. Specifically, the reduced-soy feed provided breasts with a significantly ($P < 0.01$) lower pH. On the contrary, the feed supplied to roosters and hens did not significantly ($P > 0.05$) affect any of the color parameters (Table 2). The same trend of no significant ($P > 0.05$) effect was found for WHC and shear force, as the diet did not appear to affect these breast quality parameters (Table 2).

Table 2 – Influence of reduced-soy feed on the physicochemical parameters of breast meat in the native breed 'Galiña de Mos' (values expressed as mean ± standard error).

	Males			Females		
	Control feed	Reduced-soy feed	Sig.	Control feed	Reduced-soy feed	Sig.
pH	5.90 ± 0.09	5.78 ± 0.11	**	5.85 ± 0.07	5.72 ± 0.02	**
Color parameters						
L*	53.58 ± 3.70	58.00 ± 4.92	ns	57.05 ± 1.47	54.81 ± 0.75	ns
a*	-0.51 ± 0.53	-0.76 ± 1.28	ns	0.28 ± 1.63	-0.99 ± 1.69	ns
b*	11.81 ± 2.11	11.46 ± 1.70	ns	13.01 ± 1.80	10.35 ± 1.68	ns
Water holding capacity (%)	15.85 ± 2.26	13.38 ± 2.45	ns	16.81 ± 2.23	14.35 ± 1.95	ns
Shear force (N/ cm^2)	13.29 ± 1.98	20.98 ± 8.86	ns	25.80 ± 9.67	20.09 ± 7.61	ns

Sig: significance: * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$); ns: no significant difference

IV. CONCLUSION

The implementation of reduced-soy feed for the native 'Galiña de Mos' breed yielded insignificant alterations in both the chemical composition and physicochemical parameters. This underscores the availability of sustainable alternatives to soy, mitigating its adverse environmental impacts in feed formulation, without compromising meat quality.

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Proteomic analysis reveals change in the lipid metabolism of muscle in beef cattle submitted to early weaning and pasture finished

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I. INTRODUCTION

Early weaning and postnatal nutritional management are crucial strategies in livestock production systems and can affect long-term beef cattle growth [1]. This study aimed to evaluate the effect of early weaning associated with a post-weaning supplementation on the muscle proteome of cattle pasture finished system.

II. MATERIALS AND METHODS

This experiment was approved by the Ethics and Animal Welfare Committee of Sao Paulo University (CEUA 0190/2020). Forty male Nellore calves were submitted into one of two treatments: early weaning (EW) – 120 days; and conventional weaning (CW) 205 days. The EW group was supplemented with 1.5% of BW of commercial concentrate diet until 205 days. On 205 days, both groups were combined in a single group and fed 0.3% protein + energy supplementation for 488 days. During the finishing phase (222 days) all the animals were raised on pasture and supplemented (0.5% BW). All animals were subjected to *Longissimus thoracis* muscle biopsy 24 hours before slaughter. Procedures for proteomics analysis have been previously described by Osorio [2]. Protein identification and quantitation were performed by nanoLC-MS/MS using an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). Raw data were processed using the Proteome Discoverer software (Thermo Scientific, Bremen, Germany. Protein identification analysis was performed using the UniProt protein sequence database data for the *Bos Taurus* Proteome. The number of unique peptides was set to two (minimum) per protein identified and each protein had to be identified in at least half the samples in each experimental condition. Imputation of missing data was applied to normalized and clean data using KNN method and differentially abundant proteins (DAP) were prospected using Fold Change ($FC < 0.67$ or $FC > 1.5$) and significance levels of Empirical Bayesian Test ($p\text{-value} < 0.05$) using *DEP* [3] package in R. The functional KEGG pathways enrichment was performed by over-representation analysis (ORA) from the list of DAP using *enrichR* [4] and *ClusterProfiler* [5] packages in R.

III. RESULTS AND DISCUSSION

A total of 2784 protein identifications were detected in the muscle *Longissimus thoracis* of Nellore following the LC-MS analysis, and 1546 proteins were selected after filtration. There were 118 differentially abundant proteins (DAP) between EW vs. CW, where 62 proteins were up regulated in the EW treatment. The results are presented in Figure 1A. These include protein such as Short-chain specific acyl-CoA dehydrogenase (ACADS), and Long-chain specific acyl-CoA dehydrogenase (ACADL). On the other hand, other proteins such as Carnitina O-palmitoiltransferase 1 (CPT1B) and, Integrin-linked protein kinase (ILK) were down-regulated in the EW treatment.

Regarding the DAP´s an R analysis was conducted, which found that treatments impacted mainly in the pathways such as Biosynthesis Fatty acid degradation. The interaction network between the DAPs indicated that the proteins CPT1B, ILK as associated with PPAR signaling pathway. The CPT1B is a gene member of the CPT family that is related to β-oxidation [6]. In addition, the ACADS and ACADL were associated with Fatty acid metabolism and Fatty acid degradation. The ACADS is a member of the acyl-CoA dehydrogenase family of enzymes, and it is related to maintenance of energy homeostasis by β-oxidation [7].

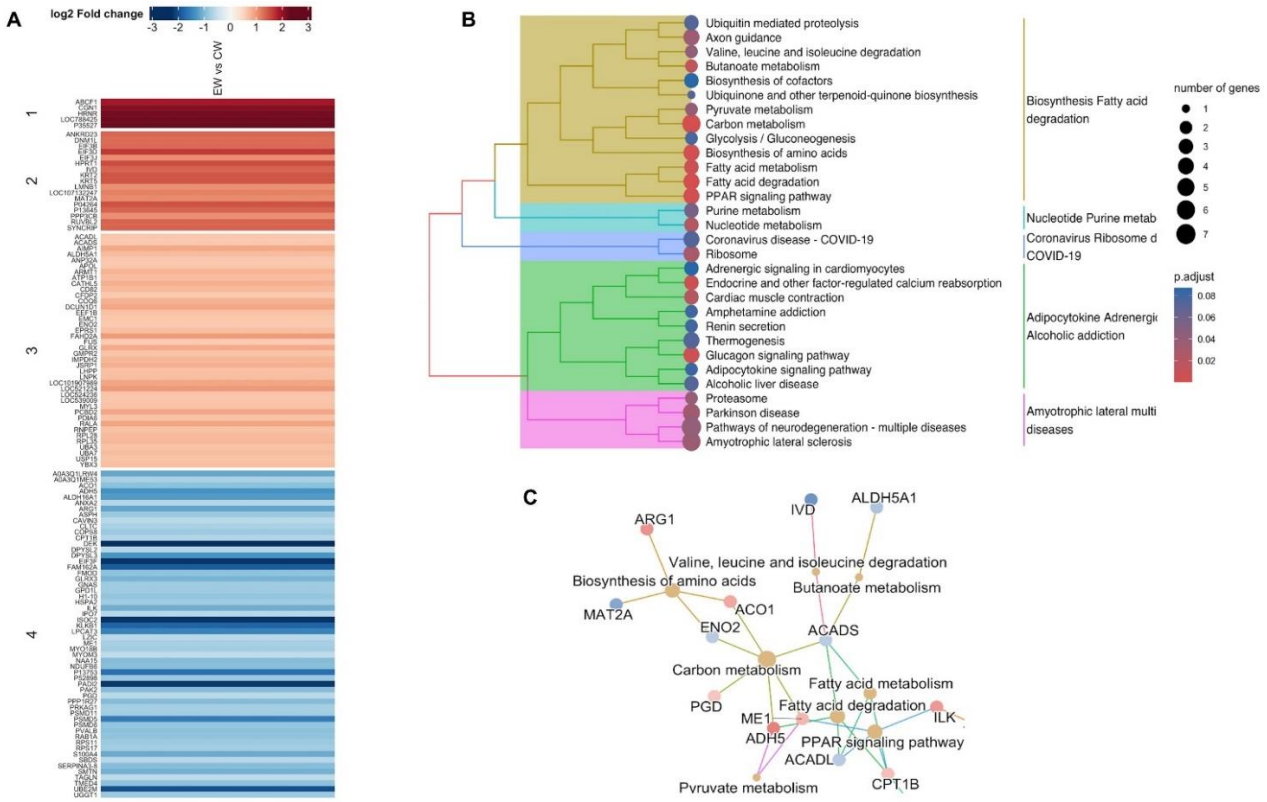

Figure 1. (**A**) Heatmap of differentially abundant proteins. (**B**) Heatmap of enriched terms obtained for the differentially abundant proteins obtained in the comparisons between EW vs. CW. (**C**) Protein interaction network content de most of differentially abundant proteins in the *Longissimus thoracis* muscle related to first pathways cluster (beige) between EW vs. CW.

IV. CONCLUSION

The present study indicates that early weaning alters the *Longissimus thoracis* proteome of cattle pasture finished system. Our data suggest that this strategy acts directly on the abundance of proteins related to the lipid metabolism pathway. The early weaning presented down regulated protein related to β-oxidation but also protein related to fatty acid degradation.

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GENETIC PROGRESS SIGNIFICANTLY IMPACTS GREENHOUSE GAS EMISSION INTENSITIES IN GLOBAL PORK PRODUCTION

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I. INTRODUCTION

The environmental sustainability of food production systems, including net greenhouse gas (GHG) emissions, holds increasing significance in defining dietary recommendations. Therefore, to maintain a portion of animal-based foods in these recommendations, it's imperative to further mitigate the net GHG emission intensities from animal husbandry systems. Pigs, as monogastric animals, exhibit lower enteric methane (CH_4) production compared to ruminants. The primary sources of net GHG emissions in pig production systems stem from manure storage, encompassing nitrous oxide (N_2O) and CH_4, as well as feed production, which includes both N_2O and carbon dioxide (CO_2). Genetic selection has resulted in improvements in reproductive and growth performance while selecting for more robust animals to minimize mortalities. Genomic selection has assisted in improving the accuracy of these traits. Since the integration of genomic selection into the pig breeding program in 2014 [1], advancements in animal performance have been significantly augmented. The aim of this study was to determine whether a model based on Integrated Pollution Prevention and Control (IPCC) methodology could accurately represent the reductions in GHG emissions resulting from genetic advancements in pork production.

II. MATERIALS AND METHODS

The GHG estimates encompass various emissions components within pork production. These include enteric and manure storage CH_4, as well as direct and indirect N_2O emissions from pig-barns and manure (in buildings and storage). Additionally, emissions from feed production, whether domestic or imported, comprise estimates for direct and indirect N_2O emissions from both manure and synthetic fertilizer, soil carbon change (CO_2), and direct and indirect energy use (expressed as CO_2 equivalents (eq.). Furthermore, the GHG emissions intensity estimates encompass GHG emissions from the production process and transportation of purchased feeds, as well as GHG emissions resulting from direct energy use in pig-barns. All stages of the pig's life, including sow and replacement of gilts, are accounted for in the analysis. GHG emissions are standardized as CO_2 equivalents to consider the global warming potential of the respective gases over a 100-year time horizon: CH_4 kg × 25 + N_2O kg × 298 + CO_2 kg × 1 [2]. The IPCC methodology-based model has been crafted and integrated into a GHG calculator tailored for pig farmers [3]. Minor adjustments to this model enable the calculation of the impact of traits selected for within the genetic program. Genetic trends spanning from 2014 to 2023 were derived using genomic estimated selection values from selection candidates across four breeds, encompassing fourteen traits. The impact of these fourteen parameters on GHG emissions was evaluated based on their respective marginal economic value (MEV) concerning total feed consumption and the volume of manure produced.

III. RESULTS AND DISCUSSION

The model facilitated the estimation of impacts on net GHG emission intensities by leveraging available production data from pork production, as outlined in Table 1.

Table 1. GHG emissions presented with percentage distribution

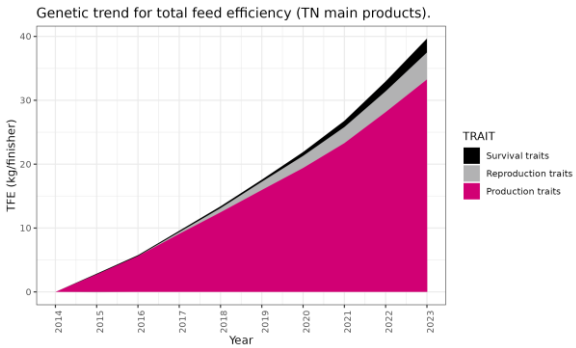
Sources and type of GHG emissions	Percentage distribution
Feeds	81%
Electricity and Transport	4%

Enteric CH_4	6%
Manure CH_4	5%
Manure N_2O	4%

In this study, we exclusively present the percentage distribution to emphasize that a substantial percentage (96%) of emissions from pigs to the farm gate originate from feed and manure. Emission levels vary among farms and countries [4]. Some nations have heightened emissions due to high-emission feed, while others may face significant emissions from manure management systems such as large lagoons. Nevertheless, in most cases, the combined influence of feed and manure governs emissions in pig production, directly impacted by the quantity of feed consumed and the efficient utilization of its nutrients, particularly nitrogen.

Among the parameters investigated, the feed conversion ratio (FCR) for finishers emerged as the most crucial. Further parameters were examined, and the fourteen parameters with the most significant effects include growth and feed intake in boar tests, drip loss, mothering ability, total born piglets, sow longevity, vitality, rear-to-finisher mortality, stillborn piglets, piglet growth, carcass yield, farrowing rate, interval between weaning and first insemination, and wean-to-rear mortality. These parameters are ordered based on the importance of the genetic progress of each trait on GHG emission intensities over the last nine years.

Figure 1 - The aggregation of 14 traits within the Topigs Norsvin selection program results in an enhancement of approximately 40 kg in total feed efficiency (TFE), leading to reduced feed consumption per finisher over a span of 9 years.



The effects of estimated genetic gain on total feed efficiency for the 14 parameters are depicted in Figure 1. Correspondingly, the improvements resulting from estimated genetic gain led to an annual reduction of 4.4 kg of total feed efficiency (TFE) per finisher. Considering a world average of 6 kg of CO_2 eq. per kilogram of carcass weight [4], this translates to a yearly reduction of 7.1 kg of CO_2 eq. per finisher.

IV. CONCLUSION

Over the past decade, genetic advancements have played a crucial role in reducing greenhouse gas emission intensities in global pork production. Feed and manure account for the largest impact on emissions and thus focus should be on traits like FCR to reduce emissions.

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BEEF-ON-DAIRY HEIFERS GRAZING SEMI-NATURAL GRASSLANDS CAN PRODUCE TENDER BEEF

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I. INTRODUCTION

Swedish consumer´s demand for Swedish beef is increasing, and so is Swedish beef production. The aim was therefore to contribute to this trend by developing innovative ways to satisfy the market with beef of high and uniform eating quality. It is well known that eating quality of beef is highly related to the degree of marbling, i.e. intramuscular fat, which is a strong predictor of eating quality as it correlates positively to juiciness, tenderness, flavour, and overall acceptability [1].

A beef cattle body becomes fatter with increasing weight and age. As the growing animal deposits fat in a predetermined order, starting with filling up the abdominal and subcutaneous fat stores, i. e. trim fat, before depositing intramuscular fat, it is almost impossible to obtain a high amount of intramuscular fat (IMF) in the meat without the carcass getting too fat. These excessive fat deposits are a waste for the processors, a cost for the farmer, and an unnecessary environmental impact from a too long animal rearing period. The aim of this study was therefore to investigate the effects of dam breed, sire breed, and intensity of production system on meat quality characteristics from dairy × beef heifer production based on forage and a well-managed semi-natural grassland.

II. MATERIALS AND METHODS

The experiment was conducted at the SLU Götala Beef and Lamb Research Centre, Skara, Sweden. During indoor periods, the animals, consisting of 72 dairy × beef heifers acquired from commercial farms, were kept in groups of six in pens with deep straw bedding, while during grazing periods all animals grazed semi-natural grassland. The animals were followed from weaning to slaughter in an experiment with a 2 × 2 × 2 factorial design, comparing two sire breeds (Angus (ANG) and Charolais (CHA)), two dam breeds (Swedish Red (SRB) and Swedish Holstein (HOL)) at two production systems: moderately high (H) and Low (L) indoor feed intensity. In addition, the two production systems also differed in terms of slaughter age and number of summers on grass. The two systems were chosen to reflect possible rearing strategies combining grazing for nature conservation and production of market-oriented carcasses. The heifers were slaughtered at an age of 27 months (L), or at 20 months (H). For details regarding diet composition and production response, see [2]. After slaughter, carcass conformation and fat cover were graded according to the European Union Carcass Classification Scheme EUROP. Marbling was determined visually between the 10th and 11th ribs in the loin using the Swedish 5-point scale from no marbling to slightly abundant. pH_{24h} was measured between the 10^{th} and 11^{th} rib. Ultimate pH was measured at 48 h post-mortem. Colour was measured using a CM-600d Konica Minolta spectrophotometer. Tenderness was measured as WBSF; samples, aged for 5 days at 4℃, were weighed, vacuum packed, and heated in waterbath at 62°C and reweighed for cooking loss. The maximum force was recorded. For IMF samples were extracted and the IMF percentage was determined from the amount of fat extracted relative to the sample weight. For details, see [3].

III. RESULTS AND DISCUSSION

Generally, beef from production system L was darker, redder, tougher, and had lower IMF percentage compared to H. Beef from ANG crossbreeds was more red and tender and higher IMF% compared with beef from CHA crossbreeds.

Table 1 Technological meat quality of *M. longissimus lumborum* from heifers with the effects of feeding intensity (moderately high and low), sire breed (Angus and Charolais) and dam breed (HOL—Swedish Holstein; SRB—Swedish Red-and-White). Results are presented as least-squares means, standard error of the mean (SEM) with P-values.

Feeding intensity	High				Low				SEM	P-values[1]		
Sire breed	Angus		Charolais		Angus		Charolais					
Dam breed	HOL	SRB	HOL	SRB	HOL	SRB	HOL	SRB		FI	S	D
n	9	9	9	9	9	9	9	9				
pH_{24}[2]	5.49ab	5.47ab	5.53ab	5.53ab	5.46a	5.59b	5.59ab	5.61b	0.03	0.014	0.007	0.104
pH_{48}[2]	5.39ab	5.40abc	5.37a	5.39ab	5.48e	5.42bcd	5.45de	5.44cde	0.01	<0.001	0.362	0.153
L*[3]	31.0abcd	32.1cd	31.5bcd	32.7d	28.4abc	28.0ab	28.0ab	27.4a	0.8	<0.001	0.958	0.411
a*[3]	15.1abc	14.8abs	14.2ab	13.8a	16.5cd	17.2d	16.0bcd	16.0bcd	0.4	<0.001	0.003	0.882
b*[3]	15.6	16.2	15.9	15.4	15.9	16.8	15.1	15.6	0.5	0.810	0.068	0.249
Cooking loss (%)	15.1	15.3	15.8	16.2	14.9	15.4	16.2	17.0	0.8	0.697	0.051	0.297
WBSF (N)[4]	38.0a	34.9a	40.1a	41.9ab	43.3ab	45.3ab	56.4b	43.3ab	3.2	<0.001	0,01	0,247
IMF (%)[5]	6.37bc	7.28c	3.96ab	4.04ab	5.27abc	5.14abc	2.80a	3.77ab	0.67	0.012	<0.001	0.326

[1]a-c: values within a row with different superscripts differ significantly at $p < 0.05$. [2]pH measured at 24 and 48 h post-mortem. [3]L* (lightness), a* (redness), b* (yellowness). [4]WBSF peak force. [5]Intramuscular fat concentration.

IV. CONCLUSION

Beef-on-dairy heifers reared on forage and semi-natural grasslands with one or two grazing seasons can deliver high quality beef. Beef from heifers reared under moderately high feeding intensity was less tough and had higher IMF% but was lighter and less red compared with heifers reared under low feed intensity. Beef from Angus crossbreeds was redder (b*) with lower shear force (WBSF), i.e. more tender and had a higher intramuscular fat content (IMF%) than Charolais. These characteristics are generally associated with superior meat quality. Notably, beef from Charolais crossbreeds in low feed intensity production system (L) was of considerably lower quality, compared to the better-performing Angus crossbreeds and Charolais crossbreeds in the moderately high production system (H). While sire breeds differed on some important meat quality traits, meat quality of Swedish Holstein (HOL) and Swedish Red (SRB) crossbreeds was comparable.

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PERFORMANCE, CARCASS TRAITS, AND MEAT QUALITY OF NELLORE CATTLE SUBJECTED TO DIFFERENT POST-WEANING FEEDING STRATEGIES

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I. INTRODUCTION

Cattle nutrition and growth after birth have a great influence on performance and body composition during the post-weaning growing phase. The backgrounding phase in Brazil occurs mainly in grazing systems and alters the quantity and quality of the meat that is produced at finishing stage [1, 2, 3]. The current study aimed to evaluate the performance, carcass traits, and meat quality of Nellore cattle subjected to different post-weaning feeding strategies. It was hypothesized that certain feed strategies offered during the post-weaning phase, before the finishing diet, would exert a higher or lower influence on carcass and meat quality.

II. MATERIALS AND METHODS

A total of 36 young Nellore bull calves, averaging 295.6 ± 8.05 kg of body weight and 7.0 ± 1 months of age, were used in this study. The calves were randomly selected to one of four treatments in the backgrounding and finishing phases: 0 + 140 days; 28 +112 days; 56 + 84 days; 84 + 56 days, respectively. The diets were formulated according to BR-CORTE recommendations to achieve average daily gains of 0.6 kg/day and 1.2 kg/day for backgrounding and finishing phase, respectively. During the experimental period, initial body weight (iBW), final body weight (fBW), and average daily gain (ADG) were measured. After harvest, data on hot carcass weight (HCW), cold carcass weight (CCW), and carcass yield (CY) were obtained. *Longissimus lumborum* (LL) samples were collected for meat quality analysis. The subcutaneous fat thickness (SFT) was measured in the *Longissimus lumborum* (LL) muscle using a digital caliper, and the Warner-Bratzler shear force (WBSF) was determined following American Meat Science Association guidelines [4]. Sarcomere length (SL) was estimated according to the laser diffraction technique [5]. The myofibrillar fragmentation index (MFI) was assessed by measuring the turbidity of homogenized samples in a standardized protein concentration [6]. Analysis of variance (ANOVA) was performed to evaluate the effect of main factors, using the GLM procedure of SAS. Once detected significant effect ($P \leq 0.05$) the treatments were compared by Tukey's test. Also, tendency was assumed when $0.05 < P \leq 0.10$.

III. RESULTS AND DISCUSSION

There was a difference in fBW (P = 0.002), ADG (P < 0.001), HCW (P < 0.001), CCW (P = 0.001), CY (P = 0.012), and MFI (P = 0.019) among treatments (Table 1). In addition, a strong tendency was observed for SFT (P = 0.055; Table 1). For all these variables, the animals that received directly the finishing diet had improved carcass and meat quality than animals that received 84 days with a high-forage diet in the post-weaning growing phase. This effect can be due to the longer confinement period when compared to other periods. This would justify using a strategy of placing the animals directly in the feedlot after weaning, optimizing space, and intensifying the production system.

Table 1 – Performance, carcass traits, and meat quality of Nellore cattle subjected to different post-weaning feeding strategies.

	Post-weaning growing phase - days with high-forage				
Variables	0	28	56	84	*P- value*
Feedlot days	157.33±2.87^{A}	157.67±2.59^{A}	157.89±2.61^{A}	158.11±2.80^{A}	0.569
iBW	265.78±30.44^{A}	264.67±28.03^{A}	265.72±31.22^{A}	263.83±29.57^{A}	0.892
fBW	442.55±48.42^{A}	417.44±32.52^{A}	408.50±50.20^{A}	361.39±46.67^{B}	0.002
ADG	1.12±0.17^{A}	0.97±0.08^{B}	0.90±0.19^{B}	0.62±0.15^{C}	<0.001
HCW	274.57±30.23^{A}	257.64±20.74^{A}	249.32±31.55^{A}	216.77±24.93^{B}	<0.001
CCW	271.44±30.22^{A}	254.67±20.42^{A}	246.83±31.10^{A}	214.21±25.00^{B}	0.001
CY	61.35±1.55^{A}	61.00±1.10^{A}	60.40±1.24^{AB}	59.37±1.42^{B}	0.012
SFT	4.44±2.65^{A}	3.10±1.33^{A}	3.79±2.59^{A}	2.47±1.06^{B}	0.055
WBSF	5.50±1.62^{A}	4.6±0.64^{A}	4.76±1.33^{A}	4.74±1.12^{A}	0.263
SL	1.28±0.19^{A}	1.29±0.16^{A}	1.19±0.05^{A}	1.32±0.33^{A}	0.802
MFI	23.69±1.02^{A}	21.46±3.24^{AB}	19.87±2.54^{B}	19.±3.75^{B}	0.019

iBW: Initial body weight (kg); fBW: Final body weight (kg); ADG: Average daily gain (kg); HCW: Hot carcass weight (kg); CCW: Cold carcass weight (kg); CY: Carcass Yield (%); SFT: Subcutaneous fat thickness (mm); WBSF: Warner-Bratzler Shear Force (KgF); SL: Sarcomere length (µm) MFI: Myofibrillar fragmentation index (%).
$^{A, B, C}$: For each variable, within a row, means without a common superscript letter are significantly different. Significant differences at 5% probability ($P \leqslant 0.05$). Tendency was assumed when $0.05 < P \leq 0.10$.

IV. CONCLUSION

These results reveal that bulls receiving a finishing diet after weaning, fed longer on a high-grain diet, have higher performance, higher carcass traits, and greater potential for producing higher meat quality than Nellore cattle subjected fed longer on a high-forage diet. Maintaining animals in a feedlot diet during the backgrounding phase may be an alternative method to obtain heavier animals at slaughter with greater, carcass traits and meat quality.

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PRODUCTION TRAITS IN NELORE CATTLE CLASSIFIED BY RESIDUAL FEED INTAKE AND DEGREE OF DAILY FLUCTUATION IN DRY MATTER INTAKE

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I. INTRODUCTION

Herds classified as efficient by residual feed intake (RFI) can reach productive indices similar to those obtained for the non-efficient ones, with lesser feed intake. This fact reflects the lower demand for environmental resources and provides greater profitability to the beef production system [1,2]. However, the continuous feed supply for “free choice” intake of confined beef cattle in Brazil may result in daily variations in dry matter intake (DMI). According to Pereira et al. [3], daily fluctuation in DMI in cattle can increase ruminitis scores, leading to metabolic disorders that can negatively affect animal development. Therefore, the present study seeks to determine whether animals classified by RFI present different degrees of daily fluctuation in DMI, causing changes in performance traits and organ size in Nelore cattle.

II. MATERIALS AND METHODS

A total of 107 non-castrated 15-month-old Nellore males were confined for 92 days, following the distribution of a completely randomized design, in 2 x 2 factorial arrangement: two groups of DMI fluctuation (GF of DMI: high or low fluctuation) and two RFI groups (negative: RFI<0; or positive: RFI>0). The fluctuation was calculated by the difference between DMI of the previous and the current day [%F-DMI = ((DMIPrevious - DMICurrent) *100)/ DMIPrevious], expressed as percentage. Then, animals were classified as high (H: 1.89%) or low (L: 0.90%) DMI fluctuation (P=0.001). The RFI was calculated using regression equations as a function of metabolic body weight, DMI, and daily weight gain (ADG). Then, animals were classified as negative or positive RFI. The production traits, hot carcass weights (HCW), carcass yield (CY), liver, kidneys, and kidney, pelvic and heart (KPH) fat weights were analyzed by PROC MIXED of SAS considering the fixed effects (GF and RFI), covariate (age at slaughter), and the random effect (year). The differences between means were compared using the Tukey test (P<0.05), and trends were verified when P<0.10.

III. RESULTS AND DISCUSSION

No differences were detected for initial and final body weight (IBW; FBW), ADG, HCW, liver, and KPH between GF or RFI groups. Animals classified as negative RFI had lower kidneys weight compared to the ones classified as positive RFI (P=0.034). There was a tendency towards lower feed efficiency ratio (FER: relationship between DMI and ADG) when comparing negative RFI animals to the positive RFI ones (P=0.088). As expected, low-DMI fluctuation animals differed from high-DMI fluctuation animals (P=0.001).

Significant interactions were detected between GF and RFI groups for DMI, CY, and RFI (Table 1). In high and low DMI fluctuation groups, the lowest dry matter intakes were detected for negative RFI animals. Animals classified as low-DMI fluctuation and negative RFI and animals classified as high-DMI fluctuation and positive RFI had the highest CY. As expected, animals classified as negative RFI were more efficient than animals classified as positive RFI. Animals

classified as high-DMI fluctuation and positive RFI were less efficient than animals classified as low-DMI fluctuation and positive RFI. This determines the effect of fluctuation in DMI in the animals' efficiency indexes.

Table 1. Productive characteristics of Nelore cattle classified by groups of DMI fluctuations and RFI

	GF		RFI		P-value		
	Low	**High**	**Positive**	**Negative**	**GF**	**RFI**	**GFxRFI**
DMI fluctuation, %	0.90a	1.89b	1.37	1.43	0.001	0.494	0.290
RFI, kg/d	-0.005	0.051	0.666	-0.619	0.626	0.001	0.017
IBW, kg	344	342	344	341	0.759	0.689	0.449
FBW, kg	486	472	478	480	0.136	0.791	0.867
DMI, kg/d	9.45	9.59	10.1	8.96	0.624	0.001	0.066
ADG, kg/d	1.49	1.52	1.54	1.47	0.472	0.228	0.710
FER, kg/kg	0.16	0.16	0.16b	0.17a	0.920	0.088	0.954
HCW, kg	285	275	280	281	0.103	0.824	0.578
CY, %	58.3	58.2	58.3	58.3	0.737	0.785	0.002
Liver, kg	5.34	5.25	5.36	5.23	0.440	0.282	0.673
Kidneys, kg	0.85	0.82	0.86a	0.81b	0.376	0.034	0.768
KPH, kg	10.84	10.52	11.05	10.31	0.495	0.194	0.301

Significant interactions

	RFI	**GF**	
		Low	High
RFI, kg/d	Positive	0.50bA	0.83aA
	Negative	-0.51aB	-0.73aB
DMI, kg/d	Positive	9.87Aa	10.28Aa
	Negative	9.04Ba	8.89Ba
CY, %	Positive	58.05Bb	58.60Aa
	Negative	58.64Aa	57.85Bb

Means followed by different letters in each row or columns differ by Tukey test (P<0.05 or trend P<0.10);
GF: DMI fluctuation groups; RFI: residual feed intake; IBW: initial body weight; FBW: final body weight; DMI: dry matter intake; ADG: average daily gain; FER: feed efficiency ratio; HCW: hot carcass weight; CY: carcass yield; KPH: kidney, pelvic and heart fat.

IV. CONCLUSION

Nelore cattle classified as efficient based on RFI consume less food do not negatively impact other traits of economic importance for beef cattle production system. Furthermore, there is an association between negative RFI animals and low DMI fluctuation animal, which provides high CY with lower DMI.

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CARCASS TRAITS OF PIGS FED WITH DDGS-ENRICHED DIETS

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I. INTRODUCTION

In the current global population scenario and, especially in the future, the rational use of resources and the maximum utilization of agro-industrial waste are becoming essential. As one of the largest producers and exporters of products from various vegetable crops and animal origin proteins, Brazil faces increasing pressure to seek the best possible alternatives to minimize environmental and social costs throughout all production cycles. Due to its volume and nutritional characteristics, distiller's dried grains with solubles (DDGS) have been commonly used in the U.S. as feedstuff for pigs and cattle. Since this practice is not yet common in South America, we evaluated the effects of increasing levels of DDGS in corn/soybean meal-based diets on pig carcass characteristics.

II. MATERIALS AND METHODS

The study registered under FZEA CEUA nº 6260270223 involved 500 piglets (250 gilts and 250 immunocastrated males) on a commercial farm, with an average age of 23 days and weight of 6.5 kg at the trial start, which were distributed in randomized blocks according to sexual condition and initial weight. Five treatments were administered to ten replicate pens, each with ten animals, over a 147-day experimental period, covering the nursery, growing, and finishing phases. For T1, DDGS was not included (T1: 0%) in the corn/soybean meal-based diet. For T2 to T5, DDGS was included from 14 days and maintained until the trial's end, replacing corn and soybean meal in the isoenergetic diets, according to the following proportions: T2: 10%; T3: 20%; T4: 30%; and T5: 40%. To evaluate carcass traits variables, two pigs weighing closest to the average weight of the pen, per experimental unit, were slaughtered. After weighing the hot carcasses, it went through a pork carcass typing probe (Hennessy© Grading Systems GP4/BP4, DIDAI), the loin depth, the backfat thickness, and the lean meat content in the carcass were evaluated at the height of the last rib, six centimeters from the carcasses midline cut. Subsequently, the carcasses were cooled for 24 hours and then weighed again. The effect of treatments was analyzed using regression with the REG procedure. Data were subjected to linear and quadratic regression analyses to determine the optimal level of DDGS inclusion. Differences between mean values were considered statistically significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

Table 1 presents the carcass traits. With increasing DDGS inclusion, a decreasing linear effect was observed on final live weight ($P < 0.0001$), hot carcass weight ($P < 0.0001$), cold carcass weight ($P < 0.0001$) and backfat thickness ($P = 0.042$). Although there was a decrease in backfat thickness, no differences were observed for loin depth or the carcass yield variables. The high neutral detergent fiber (NDF) content in DDGS may explain the weight differences between animals, as fiber increases intestinal thickness, weight and filling [1], potentially affecting yield due to reduced carcass weight during evisceration. However, these differences were not observed in this study. The changes observed are attributed to pigs receiving diets with higher DDGS inclusions becoming lighter at the end of the finishing phase, possibly due to lower daily weight gain, as indicated by previous studies. The high NDF content in DDGS can also impair digestibility [2], leading to a lighter final live weight.

Given that muscle and body weight gains are more pronounced as the finishing phase progresses [3], researchers have suggested including DDGS in the early periods of this phase and transitioning to a low-fiber diet as the finishing phase progresses to reduce undesirable carcass results [4].

Table 1 – Effects of experimental treatments on carcass traits

Traits	Treatments (DDGS inclusion, replacing corn/soybean meal)					SEM	P value	
	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	T5 (40%)		Linear	Quadratic
FLW[1], kg	130.6	130.3	127.6	126.8	123.1	2.28	**<0.0001**	0.195
HCW[2], kg	93.97	93.47	91.55	90.67	87.51	1.34	**<0.0001**	0.216
HCY, %	71.74	71.72	71.73	71.51	71.04	0.53	0.160	0.414
CCW[3], kg	91.61	91.00	90.15	88.48	85.17	1.44	**<0.0001**	0.063
CCY, %	69.94	69.84	70.27	69.79	69.14	0.47	0.137	0.276
WLDC, %	2.50	2.82	2.03	2.40	2.67	0.32	0.976	0.995
LD, mm	60.56	59.96	59.30	60.24	56.80	1.69	0.231	0.730
BT[4], mm	14.03	14.80	13.74	14.24	12.72	1.22	**0.042**	0.125
LM, %	57.41	56.80	57.41	57.21	57.71	0.84	0.472	0.409

SEM: standard error of the mean; FLW: final live weight; HCW: hot carcass weight; HCY: hot carcass yield; CCW: cold carcass weight; CCY: cold carcass yield; WLDC: weight loss during chilling; LD: loin depth; BT: backfat thickness; LM: lean meat content in the carcass. [1]Significant linear regression: y = 131.38 - 0.1852 x, R^2 = 0.9221; [2]Significant linear regression: y = 94.5953 - 0.1579x, R^2 = 0.9302; [3]Significant linear regression: y = 92.3855 - 0.1549x, R^2 = 0.8892; [4]Significant linear regression: y = 14.5569 - 0.03230x, R^2 = 0.4285.

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IV. CONCLUSION

An increasing incorporation of DDGS in pig diets has negatively impacted weight-related carcass traits. While DDGS is being tested for potential benefits, such as reduced cost or other sustainability aspects, its high-dose use in pig diets compromises important carcass traits, adversely affecting productivity.

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SESSION 3

Animal Welfare

Monday 19 August 2024

DRUG-LIKE INHIBITORS TARGETING BACTERIAL PROTEINS FOR TREATING BACTERIAL INFECTIONS OF ANIMAL FEEDS

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I. INTRODUCTION

There is an increasing threat of communicable diseases, particularly those of bacterial origin, due to the emergence of drug-resistant strains. It emphasizes the need for novel antibacterial agents, with a focus on natural products like *Rheum palmatum*, known for its various medicinal properties, including antibacterial effects. While the antibacterial activity of *R. palmatum* extracts is known [1], information on specific active constituents and their mechanisms of action is limited. To this end, development of structure-based drug discovery platforms is necessary, particularly computer-aided drug design (CADD), to screen phytoconstituents and assess their inhibitory effects against various bacterial protein targets, including penicillin-binding proteins (PBPs), peptide deformylase (PDF), topoisomerase IV, and methyl-coenzyme M reductase [2]. These proteins are essential for bacterial growth or replication and are potential targets for novel antibiotics. Therefore, the study aims to evaluate the binding energies of compounds from *R. palmatum* root extract with these protein targets using PyRx, a set of Autodock programs, to identify potential antibacterial agents, as well as to assess the drug-likeness of these hits based on Lipinski's Rule of Five and ADMET property analysis.

II. MATERIALS AND METHODS

Various bioinformatic tools such as PyRx 0.8, AutoDock, AutoDock Vina, FAFDrugs3 web server, ProTox, and the Protein Data Bank (PDB) were used for molecular docking and virtual screening. Small molecules from *Rheum palmatum* root extract were identified through GC-MS analysis, with compounds selected for docking experiments after energy minimization. Crystal structures of target proteins, including Penicillin binding proteins, Peptide deformylase, Topoisomerase IV inhibitors, and Methyl-coenzyme reductase, were retrieved from the PDB. Molecular docking simulations were conducted using AutoDock and AutoDock Vina in PyRx 0.8, employing the Lamarckian Genetic Algorithm for scoring function calculation. The grid map for docking calculations was centered on the target proteins, and the best drug-like compounds were selected based on higher scoring functions and interactions with the protein models. Visualization of structures was performed using PyMol. In silico ADMET prediction was carried out to assess drug likeness and pharmacokinetic properties, utilizing FAFDrugs3 web server for ADME parameter evaluation and ProTox server for oral toxicity assessment of the finalized compounds.

III. RESULTS AND DISCUSSION

This study focuses on virtual screening and molecular docking as a strategy for drug discovery, particularly targeting bacterial enzymes essential for pathogen survival. By screening compounds from *Rheum palmatum* root extract against four bacterial proteins crucial for infection, three potent drug-like molecules were identified: Ligand 29, Ligand 31, and Ligand 33. These compounds exhibited strong binding affinities to their respective target proteins, as indicated by their low binding energy scores. Molecular interactions between the ligands and proteins were analyzed, revealing hydrogen bonds and hydrophobic interactions crucial for inhibition. Furthermore, the drug-like properties of the selected ligands were assessed using computational tools, indicating good bioavailability, solubility,

and low toxicity. These compounds adhere to Lipinski's rule of five, suggesting potential oral drug delivery.

Table 1. Properties of ligands analyzed by FAFDrugs3 and ProTox server.

Ligand	MW	Oral bioavailability	Rotatable bonds	Flexibility	logP	HBD	HBA	Rings	Lipinski Violations	Solubility (mg/l)	Stereo centers
Lig 29	254.24	Good	0	0	3.53	2	4	1	0	4913.02	0
Lig 31	474.72	Good	23	0.74	10.21	0	4	1	1	204.40	0
Lig 33	390.56	Good	16	0.67	8.41	0	4	1	1	546.92	0

The study underscores the importance of computational approaches in drug discovery, especially in addressing challenges posed by antibiotic resistance. While the development of novel drugs from natural sources faces limitations in scalability and downstream processing, the compounds identified in this study show promise in inhibiting bacterial enzymes, offering potential for treating infectious diseases of bacterial origin.

IV. CONCLUSION

The study aimed to address emerging bacterial infections caused by antibiotic resistance by identifying new natural compounds with drug-like and antibacterial properties. Through computational screening of compounds from *R. palmatum*, three promising ligands were identified for their potential to inhibit bacterial enzymes. These ligands showed minimal toxicity and good bioavailability, indicating their suitability as novel inhibitors against bacterial infections. Further validation through in vitro and in vivo studies is needed to confirm their efficacy in treating bacterial diseases caused by drug-resistant strains.

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PURIFIED LIGNIN IN LAMB FEED AND ITS EFFECTS ON PERFORMANCE, CARCASS AND MEAT QUALITY

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I. INTRODUCTION

Kraft lignin is a by-product of the alkaline hydrolysis of wood in the papermaking process. This lignin contains phenolic fragments that have antioxidant activity in humans [1]. In its purified form, lignin has different biological effects from native lignin and does not present digestive limitations in ruminants and monogastric animals because it is composed of low molecular weight phenolics (125 to 155 g/mol) [2]. Kraft lignin in the diet of lambs (12.5 g/kgDM) exerted no protective effect on the rumen wall mucosa nor a positive effect on performance but increased the activity of peroxidases, indicating antioxidant activity [3].Kraft lignin can be beneficial in increasing feed conversion and animal performance, however, its use in animal nutrition needs more studies to be fully understood [4]. This study aimed to establish the best concentration of purified Kraft lignin in the diet of lambs in the finishing phase.

II. MATERIALS AND METHODS

A total of 32 lambs from industrial crossbreeding, with an average body weight of 20 kg and an approximate age of 60 days, were fed 65 days of a diet with 85% concentrate and 15% hay with 0, 6, 12 or 18 g/kg DM of purified Kraft lignin, respectively treatments L0, L6, L12 and L18. The animals were weighed in the morning every 13 days of feeding using an electronic scale. After slaughter, carcass weight was measured, as well as carcass yield, pH, temperature, loin eye area, fat thickness, TBARS, and meat from commercial cuts. The experimental design was a randomized block design with eight animals per treatment, and the statistical analysis was carried out using the SAS program, and the means were compared using the Tukey test with a probability of 5%.

III. RESULTS AND DISCUSSION

The inclusion of purified Kraft lignin in the concentrations of 6, 12 and 18 g/kg of DM had no effect on body weight, average daily weight gain, feed conversion and dry matter consumption. Furthermore, there were no changes in carcass quality parameters, hot carcass weight, cold carcass weight, hot carcass yield and cold carcass yield, loin eye area, pH and temperature (at 30 minutes and 24 hours after slaughter) and TBARS (Table 1). Also, there were no changes in the commercial cuts (Table 2). Under the conditions of this study and at the concentrations used, purified Kraft lignin did not affect performance characteristics, carcass except the fat thickness, and meat quality parameters.

Table 1 – Carcass parameters of lambs fed different amounts of purified Kraft lignin

	Treatment						
	L0	L6	L12	L18	SEM	Means	*P*-value
Hot carcass weight, kg	20.35	19.68	19.20	19.50	2.30	19.68	0.7842
Cold carcass weight, kg	19.84	19.13	18.69	19.01	2.26	19.17	0.7768
Hot carcass yield, kg	46.50	46.25	46.38	46.25	0.02	46.34	0.9936

Cold carcass yield, kg	45.25	44.75	45.00	45.00	0.02	45.00	0.9677
Subcutaneous fat, mm	1.85a	1.57b	1.40 b	1.44b	0.05	1.57	0.0340
Loin eye area, cm^2	15.47	13.91	14.52	14.05	1.82	14.56	0.3910
pH, 30 min	5.86	6.13	6.13	6.18	0.538	6.07	0.6395
T°C, 30 min	32.99	32.64	33.36	32.75	1.70	32.93	0.8361
pH, 24 hours	5.58	5.54	5.61	5.63	0.13	5.59	0.5272
T °C, 24 hours	6.05	7.46	6.80	6.11	1.48	6.61	0.2101
TBARS, µg malondialdehyde/kg	0.16	0.16	0.11	0.14	0.09	0.14	0.7096

Parameters in each row with P > 0.05 are not different. Treatments means with the same letter are no different P > 0.05.

Table 2 – Commercial carcass cuts from lambs fed different amounts of purified Kraft lignin

Meat cuts	Treatment				SEM	Means	*P*-value
	L0	L6	L12	L18			
Shank, kg	3.17	3.05	2.95	3.07	0.42	3.06	0.7738
Loin, kg	0.93	0.94	0.85	0.92	0.23	0.91	0.8575
Rib and neck, kg	4.33	4.07	3.84	3.95	0.56	4.05	0.3515
Shoulder, kg	1.79	1.79	1.77	1.78	0.22	1.78	0.9979

Parameters in each row with P > 0.05 are not different.

IV. CONCLUSION

Under the conditions of this study and at the concentrations used, purified Kraft lignin had no effect on performance, carcass characteristics and meat quality parameters when added to finishing diets of lambs, except for a slight reduction in fat thickness. Therefore, the use of this type of lignin is not recommended for feeding lambs on this type of diet, but studies with low energy diets should be carried out.

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IS RESIDUAL FEED INTAKE ASSOCIATED TO CARCASS AND MEAT QUALITY TRAITS?

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I. INTRODUCTION

Improving feed efficiency is a key factor in livestock systems to reduce feeding costs and enhance profitability [1], and it is also associated with beef production sustainability. Residual feed intake (RFI) is one of the methodologies for calculating feed efficiency of growing beef cattle, which is a moderately heritable characteristic enabling genetic improvement through selection [2]. However, literature is not entirely conclusive about the effect of RFI genetic selection on beef quality. Therefore, the aim of the present study was to evaluate the association between RFI at finishing and carcass and beef quality traits of Hereford steers.

II. MATERIALS AND METHODS

Residual feed intake of 136 Hereford steers was measured in 2 years (n = 67 in 2022 and n = 69 in 2023) at the Central de Pruebas Kiyú, San José, Uruguay. All methods and procedures used in live animals were approved by the Committee for the Ethical Use of Animals of the Instituto Nacional de Investigación Agropecuaria, Uruguay (Protocol number 2018-11). Steers were fed in a feedlot system and daily feed intake of each animal was recorded using a GrowSafe™ automated system (GrowSafe Systems Ltd., Alberta, Canada). Feed intake was adjusted by the dry matter percentage to determine the dry matter feed intake. Steers were categorized into three groups: high RFI (HRFI; >0.5 SD above the RFI mean; n = 44), medium RFI (MRFI; RFI mean ± 0.5 SD; n = 55), and low RFI (LRFI; <0.5 SD below the RFI mean; n = 37). Steers achieved an average pre-slaughter live weight (LW) of 535.8 ± 2.87 kg. At slaughter, hot carcass weight (HCW) was recorded, and dressing percentage was calculated as: ((HCW/LW) *100). After quartering ribeye area (REA, cm^2) and subcutaneous fat thickness (FAT, mm) were measured between the 10^{th}-11^{th} ribs. A 2.5 cm steak was removed from the *Longissimus thoracis* muscle between the 11^{th} to 13^{th} rib, vacuum-packed individually and transported to the Meat Laboratory of INIA Tacuarembó. Steaks were aged for 5 days and then instrumental meat color (CIE L*: lightness, a*: redness and b*: yellowness) was measured in triplicate with a Minolta colorimeter CR-400 (Konica Minolta Sensing Inc., Osaka, Japan) after 45 min blooming. Subsequently, Warner- Bratzler shear force (WBSF; model D2000- WB, G-R Electric Manufacturing Co, Manhattan, KS, USA) was assessed according to the American Meat Science Association guidelines [3]. Steaks were weighed before and after cooking and cooking losses were calculated as: ((weight of raw steak - weight of cooked steak)/weight of raw steak) x 100. The statistical model included RFI groups as fixed effects and year as a random effect. Data was analyzed using the Mixed procedure of SAS (SAS Institute, Cary, NC, USA, version 9.4).

III. RESULTS AND DISCUSSION

Steers from the three RFI groups did not differ (P > 0.05) in final live weight, hot carcass weight, dressing percentage, degree of marbling, ribeye area and subcutaneous fat thickness which agree with previous research [1] [4] [5]. However, Herd et al. [6] reported greater subcutaneous fat depth at the $10/11^{th}$ ribs on high-RFI (less efficient) Angus steers than low-RFI animals. Our findings indicate a lack of association between RFI and carcass traits. In terms of beef quality, no differences were observed among RFI groups on instrumental lean color, cooking losses and WBSF values. Blank et al. [7] did not observe differences on WBSF of beef aged for 14 days between high and low feed efficient British x Continental crossbred steers and Pravia et al. [5] did not find differences on WBSF values of beef aged for 5 days from Hereford steers neither. Nevertheless, Zorzi et al. [8] found greater WBSF values (less

tender beef) in low compared to high RFI Nellore bulls when beef was aged for 7 and 21 days. Beef color is an important characteristic affecting consumer´s purchase decision [9]. As in the study previously carried out by our research group [5], no differences were detected on L*, a* and b* coordinates of lean color among RFI groups which agree with the findings reported by Reis et al. [10] in heifers.

Table 1 – Carcass traits and beef quality characteristics by RFI group.

Traits		Treatments			Pr > F		
		HRFI	MRFI	LRFI	RFI	Year	RFI* Year
Final live weight, kg		543.6 ± 5.0	532.2 ± 4.4	534.8 ± 5.3	0.2177	0.2108	0.9353
Hot carcass weight, kg		293.9 ± 2.3	288.3 ± 2.1	290.2 ± 2.5	0.1968	0.6600	0.6219
Dressing percentage, %		54.1 ± 0.2	54.4 ± 0.2	54.3 ± 0.2	0.6860	<0.0001	0.0695
Marbling (USDA scores)[1]		499 ± 7.3	497 ± 6.5	483 ± 7.8	0.2893	0.0754	0.1958
Ribeye area, cm^2		63.3 ± 0.9	64.3 ± 0.8	66.2 ± 1.0	0.1074	0.0002	0.2142
Fat thickness, mm		14.7 ± 0.6	14.9 ± 0.5	14.8 ± 0.6	0.9608	<0.0001	0.1377
Meat Color (5 d aging)	L*	38.1 ± 0.4	38.3 ± 0.4	38.5 ± 0.4	0.8076	0.0201	0.3693
	a*	22.4 ± 0.2	22.3 ± 0.2	22.3 ± 0.3	0.9384	0.0003	0.4970
	b*	11.1 ± 0.2	11.1 ± 0.1	10.9 ± 0.2	0.7427	0.0008	0.7371
Cooking losses, %		20.4 ± 0.4	20.8 ± 0.4	20.8 ± 0.4	0.7689	<0.0001	0.0523
WBSF (5 d aging), kg		3.59 ± 0.15	3.73 ± 0.13	3.45 ± 0.16	0.3921	0.5478	0.0658

[1]: USDA marbling scores were encoded as follows: slight = 300 to 399, small = 400 to 499.

IV. CONCLUSION

The present study and previous research conducted by our team did not find any association between carcass traits and beef quality attributes and RFI in Hereford steers. Therefore, improving RFI would improve beef production economic equation reducing feeding costs with non-detrimental effect on product quality.

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IN UTERO EXPOSURE TO EARLY OR CONVENTIONAL WEANING AND DAM PARITY INFLUENCE ON CARCASS CHARACTERISTICS OF NELLORE BULLS

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I.INTRODUCTION

In tropical beef cattle production, late pregnancy is a period of increased nutritional requirements for maintenance, gestation, and lactation, which coincides with the year's dry season. Moreover, for young cows, these requirements are further intensified by the necessity to support their own growth. Thus, implementing an early weaning strategy emerges as a possible approach to alleviate the nutritional requirements of the dams, particularly for primiparous or secundiparous cows, leaving more nutrients for fetal growth.

Previous research reported that lactation during gestation decreases the embryonic and post-natal growth of the offspring in taurine cows (1,2,3). However, there are currently no reports on the effect of early weaning or parity of zebu cows on the carcass characteristics of the next offspring.

The objective of this study was to evaluate the effect of weaning either early (150 days) or conventionally (240 days) in multiparous or secundiparous cows, on the carcass characteristics of bulls that were *in utero* at the time of weaning. We hypothesized that bulls born from dams weaned early in the previous lactation, particularly from secundiparous dams, would exhibit improved carcass characteristics.

II. MATERIALS AND METHODS

This experiment was conducted at the College of Animal Science and Food Engineering (FZEA) of the University of São Paulo (USP), under the approval of the Institutional Animal Care and Use Committee (# 2884250620).

Experimental units, including primiparous and multiparous Nellore cows, were assigned to one of two weaning strategies where suckling calves were either early weaned 150 days (EW) or conventional weaned at 240 days (CW). All cows were managed similarly after weaning, and through parturition, and weaning of subsequent calves, with resulting calves managed together until use in the current experiment. Fifty-six Nellore bulls with an initial body weight of 417.4±47.63 kg and 18 months of age were allocated to feedlot in three collective pens equipped with Intergado® electronic feeders (Betim, MG, Brasil) and fed a total mixed ration (80:20 concentrate:corn silage) for an average of 97 days. The experimental design used a block (pens, n=3) incomplete randomized 2x2 factorial arrangement, considering fixed effects of dam weaning strategy (EW or CW), and dam parity at subsequent calving (multiparous or secundiparous – MC and SC, respectively).

Bulls were selected for slaughter within each block and with at least 3 mm of subcutaneous fat thickness measured by ultrasound (SFTu). The slaughter was conducted following the humane slaughter practices outlined in the Regulations for Sanitary Inspection and Industrialization of Animal Origin Products [4]. Hot carcass weight was recorded and the dressing percentage was calculated. After 24 of chill were determined the ribeye area (REA) and subcutaneous fat thickness (SFT), in the *Longissimus thoracis* muscle at the 12th rib level.

Data were analyzed using PROC MIXED of SAS 9.4, considering the fixed effects weaning strategy, dam parity and its interaction as fixed effects. Significance was considered when P values were less than 0.05 and a trend when $0.05 < P \leq 0.10$.

III. RESULTS AND DISCUSSION

No significant interaction was observed for most traits, except for SFT, that tended to show a weaning strategy × dam parity interaction (*P*=0.091). No effect of weaning strategy was found for body weight, carcass weight or dressing percentage (Table 1), however, ribeye area tended to be greater in CW group (*P*=0.083), compared to EW. Meanwhile, bulls born from MC cows had greater shrunk body weight (*P*=0.030) and hot carcass weight (*P*=0.035) than bulls from SC cows. The subcutaneous fat thickness was smaller in the SC-CW group (*P*<0.05), while other treatments did not differ. The lower SFT for SC-CW cows plus the better results for carcass characteristics of the bulls born to MC cows may be related to a developmental programming effect whereby the SC cows had greater nutritional demands due to continued growth compared with MC cows, resulting in MC cows having greater availability of nutrients for fetal growth [1].

Table 1 – Carcass characteristics of Nellore bulls from secondiparous or multiparous dams that were in utero at the time when dams were exposed to early or conventional weaning strategies.

	Weaning strategy			Cows parity			P – Value[2]		
Item[1]	Early	Conventional	SEM[2]	Multiparouss	Secundiparous	SEM[2]	W	P	W×P
Nº. Cattle	27	29	—	29	27	—	—	—	—
Age at slaughter, months	22	22	0.18	22	22	0.18	0.320	0.420	0.660
SBW, kg	573.87	570.45	8.86	585.02	559.29	8.29	0.748	0.030	0.358
HCW, kg	324.90	324.24	5.48	332.12	317.02	5.01	0.918	0.035	0.625
HCY, %	56.62	56.81	0.24	56.73	56.69	0.23	0.597	0.921	0.212
REA, mm²	77.93	81.50	1.54	79.99	79.44	1.43	0.083	0.803	0.765

[1]SBW: Shrunk body weight; HCW: Hot carcass weight; HCY: Hot carcass yield; REA: Rib eye area; SFT: Subcutaneous fat thickness.
[2]SEM: Standard error of the mean; W: Weaning effect; P: Cow parity effect; W×P: Interaction between weaning and cow category.

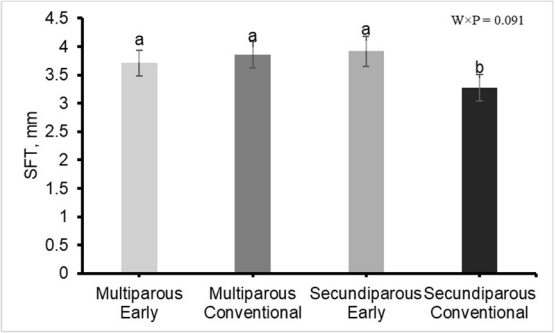

Figure 1. Interaction for subcutaneous fat thickness of Nellore bulls from secondiparous or multiparous dams that were in utero at the time when dams were exposed to early or conventional weaning strategies.

IV. CONCLUSION

In utero exposure to early weaning due to the interruption of milk production tended to improve the deposition of subcutaneous fat in bulls born from secundiparous cows. Bulls born from multiparous cows had greater carcass weight compared to those born from secundiparous cows. These results suggest the necessity that most studies use different ages and strategies for weaning in young cows (primiparous and secundiparos).

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BEEF QUALITY FROM ANIMALS RAISED IN THREE DIFFERENT GRAZING SYSTEMS

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I. INTRODUCTION

The increase in greenhouse gas emissions (GHG) is considered one of the leading causes of global warming, and enteric methane (CH_4) from beef cattle represents an important emission source in the agricultural sector [1]. In the pigeon pea (*Cajanus cajan* cv. BRS Mandarin) and *Urochloa spp.* consortium, the legume acts as a protein bank and green manure, reducing GHG emissions [2]. Intensified systems and nutritional strategies improve production efficiency and can influence meat quality. The present study aimed to evaluate the quality of aged beef produced in three pasture systems: degraded, recovered and consortium with pigeon pea.

II. MATERIALS AND METHODS

Twenty-seven *Nellore* steers, initially weighing 221±7 kg and 15-16 months of age, were distributed into three treatments with three replications: degraded pasture (*Urochloa spp.*), recovered pasture (*Urochloa spp.* fertilized with 200 kg of N/ha^{-1}) and consortium pasture with pigeon pea (*Cajanus cajan* cv. BRS Mandarin) and *Urochloa spp.*, for two years. The animals were slaughtered in a commercial slaughterhouse following standards and guidelines for the industrial and sanitary inspection of products of animal origin. After 24 hours in a cold chamber, samples of the *Longissimus thoracis* muscle (between the 12^{th} and 13^{th} rib) from the left half carcass of each animal were removed and taken to the Meat Analysis Laboratory of Embrapa Southeastern Livestock, São Carlos, São Paulo, Brazil. 2.5 cm thick steaks were aged for 14 days at 0-2 °C, and the following analyses were performed at 0, 7, and 14 days: pH; instrumental color measurement, using a portable Hunter Lab Miniscan XE colorimeter to measure variations within the CIE system color space (L*, a*, b*) [3]; water holding capacity (WHC) [4], cooking loss (CL) and shear force (WBSF) [5]. The obtained data were analyzed by analysis of variance (ANOVA) using PROC MIXED in SAS software, and mean comparisons were performed using Tukey's test (5%).

III. RESULTS AND DISCUSSION

The results obtained for the meat quality traits are presented in Table 1. Based on the analyzed data, the grazing systems had no effect ($p>0.05$). On the other side, there was a significant effect ($p<0.01$) of aging times on all the meat quality traits, except for a*.

Table 1 - Quality traits of beef produced in different pasture systems.

Effects		Variables				
Grazing systems	Aging Time (days)	Color		pH	CL (%)	WBSF (kgf)
		a*	b*			
Degraded		13.32	10.80	6.05	24.23	8.35
Recovered		13.74	10.46	6.05	25.13	7.59
Pigeon pea+*Urochloa spp.*		14.09	11.32	5.97	23.01	7.71
	0	13.81	10.61^{B}	5.96^{B}	26.77^{A}	9.34^{A}
	7	13.66	11.26^{A}	6.04^{A}	23.00^{B}	8.25^{B}
	14	13.68	10.70^{B}	6.07^{A}	22.60^{B}	6.06^{C}
Average		13.71	10.86	6.02	24.12	7.89
SEM		0.20	0.25	0.04	0.71	0.40

	Statistical Probabilities (P value)				
Treatment	0.2469	0.3420	0.6702	0.2546	0.6693
Aging time	0.5608	<.0001	<.0001	0.0003	<.0001
Treat.*AgingTime	0.3285	0.6256	0.8625	0.7061	0.9371

Lowercase letters differ among grazing systems (P≤0.05). Uppercase letters differ (P≤0.05) among aging times. CL = cooking loss; WBSF = Warner Bratzler Shear Force

A significant interaction was observed in L* (P=0.0484) and WHC (P=0.0241) of the meat produced in the pigeon pea + *Urochloa spp.* treatment at the 7-day aging time (Figure 1).

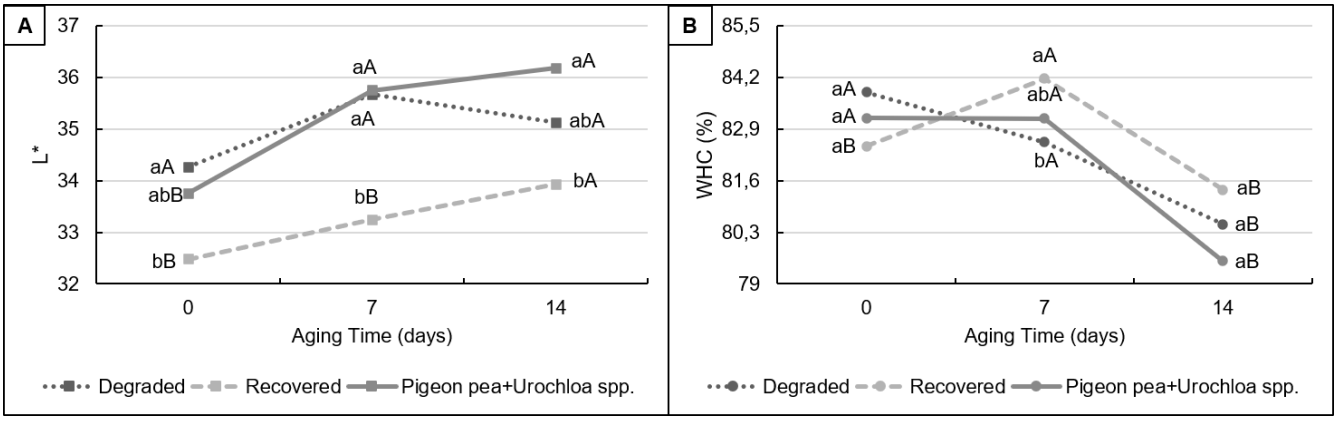

Figure 1 - Effect of the interaction between grazing systems and aging times for luminosity (L*) (A) and water holding capacity (WHC) (B) of the produced beef. Lowercase letters differ among grazing systems (P≤0.05). Uppercase letters differ (P≤0.05) among aging times.

For L*, it is observed a significant difference (p<0.05) in zero and seven days, and not at 14 days for the grazing systems (Figure 1A). For WHC (Figure 1B), the behavior between aging times and grazing systems was different: meat of animals from the recovered system showed a lower value on day zero and higher at day 7, indicating that depending on aging time, there was a difference between WHC (%) values in the grazing systems.

IV. CONCLUSION

The pigeon pea+*Urochloa spp* consortium system, in addition to being a strategy to mitigate GHG emissions, generally did not affect beef quality, except for luminosity and water holding capacity.

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EFFECT OF RUMEN-PROTECTED FAT ON GROWTH PERFORMANCE, MEAT QUALITY AND INTRAMUSCULAR FAT DEPOSITION IN FATTENING YAKS

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I. INTRODUCTION

With a population of 16 million, the yak industry provides considerable beef product for the meat consumption market in China. However, the low productive performance and poor meat quality under traditional grazing feeding pattern are currently hindering the modernization development of yak industry. Fat, as an essential nutrient for livestocks, may cause adverse impact on ruminal microbes and fermentation at high supplemental levels in a ruminant diet. Rumen-protected fat (RPF) which is coated by physical or chemical method has been reported to avoid the interference with ruminal microorganisms, meanwhile promote growth performance and milk production via the effective improvement of dietary energy. At present, there is few literatures on the application of RPF to yak ration. Therefore, the objective of this study was to investigate the effect of dietary RPF supplementation on growth performance, meat quality and intramuscular fat deposition of fattening yaks.

II. MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of the Sichuan Academy of Grassland Sciences approved all procedures in the study. Male yaks (n=24, 3-4 years old) with similar body weight (275.63±9.84 kg) were assigned to 1 of 3 treatments with completely randomized design. Yaks in different treatments received (1) basal diet (CON), (2) basal diet with 1.5% RPF supplementation (RPF1.5), or (3) basal diet with 3.0% RPF supplementation (RPF3.0), respectively. There were 8 yaks for each treatment. The experimental diets fed as TMR consisted of concentrate, corn silage and wheat straw.

After an adaptation period of 7 d and an experimental period of 90 d, all yaks were weighed. Three yaks in each treatment were randomly selected for slaughtering measurements following standard procedure, respectively. *Longissimus dorsi* samples were collected to analysis meat quality and gene expression related to intramuscular fat (IMF) deposition. Statistical analyses were performed using the one-way ANOVA procedure of SAS (SAS Institute Inc.).

III. RESULTS AND DISCUSSION

Growth Performance and Carcass Traits

Table 1. Effect of rumen-protected fat supplementation on the growth performance of fattening yaks

Items	CON	RPF1.5	RPF3.0	SEM	P-value
Initial body weight, kg	274.56	274.88	277.44	3.56	0.828
Final body weight, kg[1]	343.31^{B}	350.69^{AB}	358.50^{A}	4.03	0.048
Body weight change, kg	68.75^{B}	75.81^{A}	81.06^{A}	2.09	0.002
Average daily gain, g/d	763.89^{B}	842.36^{A}	900.69^{A}	23.20	0.002
Dry matter intake, kg	8.12	8.05	7.96	0.09	0.484
Feed to gain ratio	10.71^{A}	9.62^{B}	8.87^{B}	0.27	<0.001

[1]Values in the same row with different letter superscripts differed significantly (P<0.05).

We observed significant increase of body weight change for yaks fed RPF diets compared with those fed basal diet (P < 0.05, Table 1). Average daily gain differed significantly among treatments while yaks received 3.0% RPF supplementation showed the highest growth (P < 0.05). Yaks in RPF1.5 and RPF3.0 group had significantly lower feed to gain ratio which reveals greater feed efficiency (P < 0.05).

After slaughtering measurements, significant effect on the carcass traits occurred with dietary RPF supplementation. 1.5% and 3.0% RPF supplementation resulted in greater visceral fat weight in comparison with control group (P < 0.05). The eye muscle area, carcass weight, net meat weight, dressing percentage and net meat percentage of yaks fed RPF3.0 diet were significantly higher than those fed basal diet (P < 0.05).

Meat Quality

The cooking loss and shear force of *Longissimus dorsi* were significantly reduced by RPF3.0 treatment (P < 0.05, Table 2). And we found a significant increase of IMF content for yaks received RPF diets when compared with CON (P < 0.05). The fatty acids composition in IMF including SFA, MUFA and PUFA did not differ significantly among treatments.

Table 2. Effect of rumen-protected fat supplementation on meat quality and nutrients composition of fattening yaks

Items	CON	RPF1.5	RPF3.0	SEM	P-value
Cooking loss, %	37.61^{A}	34.13^{AB}	32.20^{B}	1.23	0.063
Shear force, kg	7.49^{A}	6.58^{AB}	5.78^{B}	0.08	0.017
Protein, %	22.17	22.07	21.83	0.42	0.861
Intramuscular fat, %	2.60^{C}	3.53^{B}	4.57^{A}	0.20	0.005
SFA[1], %	55.30	55.62	57.32	1.18	0.550
MUFA, %	41.66	42.46	40.50	1.20	0.589
PUFA, %	3.05	1.93	2.16	0.47	0.446

[1]SFA=Saturated fatty acids; MUFA=Monounsaturated fatty acids; PUFA=Polyunsaturated fatty acids.

Gene Expression Related to Intramuscular Fat Deposition

Table 3. Effect of rumen-protected fat on gene expression related to intramuscular fat deposition of fattening yaks

Items	CON	RPF1.5	RPF3.0	SEM	P-value
Acetyl-CoA Carboxylase (ACC)	1.00^{A}	0.81^{A}	0.54^{B}	0.06	0.008
Fatty Acid Synthase (FAS)	1.01^{A}	0.61^{B}	0.66^{B}	0.06	0.006
Stearoyl-CoA Desaturase (SCD)	1.02	1.10	1.18	0.08	0.539
Diacylglycerol O-Acyltransferase 1 (DGAT-1)	1.02^{C}	1.51^{B}	2.10^{A}	0.09	<0.001
Lipoprotein Lipase (LPL)	1.00	1.07	0.94	0.09	0.714
Hormone-Sensitive Lipase (HSL)	1.01	0.96	0.93	0.07	0.722
Adipose Triglyceride Lipase (ATGL)	1.03	1.19	1.03	0.15	0.756
Carnitine Palmitoyltransferase 1 (CPT-1)	1.01^{B}	1.28^{A}	1.48^{A}	0.07	0.012
Acyl-CoA Oxidase (ACOX)	1.02	1.24	1.24	0.12	0.368

The gene expression of ACC and FAS, key enzymes of *de novo* lipogenesis, was significantly lower in the RPF3.0 group than control group (P<0.05, Table 3). The DGAT-1 gene expression differed significantly among treatments, and yaks fed with RPF1.5 and RPF3.0 supplementation had significantly greater DGAT-1 expression compared with those fed basal diet (P<0.05). In contrast, no effect of treatments on the gene expression of key enzymes of lipolysis was observed, except CPT-1 which was increased significantly in RPF groups in comparison with the control group (P<0.05).

IV. CONCLUSION

The results of this study indicate that yaks fed with 3.0% RPF supplementation had improved growth performance and meat-producing capacity. Based on the reduced shear force and cooking loss, as well as elevated intramuscular fat content, the meat quality of yak could be improved by dietary RPF supplementation. The increased IMF deposition resulted from the direct utilization of the fatty acids derived from RPF for triglycerides synthesis, instead of *de novo* lipogenesis.

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BELLY QUALITY OF PIGS FED WITH DDGS-ENRICHED DIET

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I. INTRODUCTION

Brazil, a key player in agricultural and animal product exports, faces increasing pressure to adopt sustainable practices. We suggest incorporating distiller's dried grains with solubles (DDGS) into pig feeds, potentially replacing corn and soybean meal without compromising belly quality. Our study examines the impact of DDGS inclusion on belly quality attributes and lipid profile, crucial for bacon quality, given the overlap of Brazil's corn production, pig farming, and agroenergy sectors.

II. MATERIALS AND METHODS

The study registered under FZEA CEUA nº 6260270223 involved 500 piglets (250 females and 250 immunocastrated males) on a commercial farm, with an average age of 23 days and weight of 6.5 kg at the start, which were distributed in randomized blocks according to sexual condition and initial weight. Five treatments were administered to ten replicate pens, each with ten animals, over a 147-day experimental period, covering the nursery, growing, and finishing phases. For T1, DDGS was not included in the diet (T1: 0%). For T2 to T5, DDGS was included from 14 days and maintained until the experiment's end, replacing corn and soybean meal in the isoenergetic diets, according to the following proportions: T2: 10%; T3: 20%; T4: 30%; and T5: 40%. To evaluate belly quality variables, two pigs weighing closest to the average weight of the pen, per experimental unit, were slaughtered. The belly of each left half carcass was assessed for its length, width, average thickness, and flexibility [1] [2]. For belly fat quality analyses, lipid profile analysis was performed after lipid extraction [3], followed by the methyl ester preparation process [4]. The determination of the fatty acid profile was carried out by gas chromatography. The iodine value was calculated in g/100g of fat [5]. The effect of treatments was analyzed using regression with the REG procedure. Data were subjected to linear and quadratic regression analyses to determine the optimal level of DDGS inclusion. Differences between mean values were considered statistically significant at $P < 0.05$.

III. RESULTS AND DISCUSSION

Table 1 presents belly quality and fatty acid profile results. With the increase in DDGS inclusion, a decreasing linear effect was observed in belly weight ($P = 0.0002$), thickness ($P < 0.0001$), SFA ($P < 0.0001$), MUFA ($P < 0.0001$), and PUFA n3 ($P = 0.028$). Additionally, a quadratic effect was observed in external ($P = 0.021$) and internal ($P = 0.025$) belly flexibility, with the lowest external flexibility estimated at 37% DDGS inclusion and the lowest internal flexibility at 38% DDGS inclusion. An increasing linear effect was observed in PUFA ($P < 0.0001$), PUFA/SFA ($P < 0.0001$), PUFA n6 ($P < 0.0001$), n6/n3 ($P < 0.0001$), and iodine value ($P < 0.0001$). These changes are due to the high unsaturated fatty acid content in DDGS [6], as pigs, being monogastric, alter their fatty acid composition based on their diet.

Table 1 – Effects of experimental treatments on belly quality and belly fat fatty acid profile.

Traits	Treatments (DDGS inclusion)					SEM	P value	
	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)	T5 (40%)		Linear	Quadratic
Belly weight[1], kg	6.26	5.99	5.83	5.77	5.58	0.225	**0.0002**	0.595

Belly length, cm	58.2	57.5	57.5	58.4	57.9	0.733	0.855	0.566
Belly width, cm	29.6	29.6	29.6	29.8	29.3	0.335	0.738	0.515
BEF[2], cm	19.1	15.6	13.5	12.5	12.2	2.110	<0.0001	**0.021**
BIF[3], cm	27.6	23.3	20.3	19.3	18.6	2.933	<0.0001	**0.025**
Belly thickness[4], cm	3.82	3.55	3.43	3.40	3.23	0.131	**<0.0001**	0.381
SFA[5], %	35.2	34.5	33.8	33.3	33.0	55.44	**<0.0001**	0.310
MUFA[6], %	52.2	51.1	49.6	48.7	46.8	35.96	**<0.0001**	0.492
PUFA[7], %	12.1	14.2	16.4	17.9	20.4	74.64	**<0.0001**	0.921
PUFA/SFA[8]	0.35	0.41	0.49	0.54	0.63	0.018	**<0.0001**	0.792
PUFA n3[9], %	7.31	6.99	7.00	6.69	6.24	0.402	**0.028**	0.627
PUFA n6[10], %	11.06	13.05	15.17	16.6	19.1	71.20	**<0.0001**	0.799
n6/n3[11]	1.49	1.89	2.32	2.56	3.16	1.461	**<0.0001**	0.674
IV[12], g/100g	65.2	67.5	69.7	71.5	73.9	61.04	**<0.0001**	0.911

SEM: standard error of the mean; BEF: belly external flexibility; BIF: belly internal flexibility; SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; PUFA/SFA: ratio of PUFA to SFA; n3: omega 3; n6: omega 6; n6/n3: ratio of n6 to n3; IV: iodine value. [1]Significant linear regression: $y = 6.1993 - 0.01581x$, $R^2 = 0.9596$; [2]Significant quadratic regression: $y = 19.0311 - 0.3734x + 0.005045x^2$, $R^2 = 0.9985$; [3]Significant quadratic regression: $y = 27.5203 - 0.4765x + 0.006353x^2$, $R^2 = 0.9963$; [4]Significant linear regression: $y = 3.7487 - 0.01331x$, $R^2 = 0.9234$; [5]Significant linear regression: $y = 35.117 - 0.5811x$, $R^2 = 0.9788$; [6]Significant linear regression: $y = 52.287 - 1.3125x$, $R^2 = 0.9888$; [7]Significant linear regression: $y = 12.101 + 2.0502x$, $R^2 = 0.9954$; [8]Significant linear regression: $y = 0.3456 + 0.006894x$, $R^2 = 0.9965$; [9]Significant linear regression: $y = 0.73422 - 0.02457x$, $R^2 = 0.8898$; [10]Significant linear regression: $y = 11.061 + 1.9757x$, $R^2 = 0.9954$; [11]Significant linear regression: $y = 14.8394 + 0.4004x$, $R^2 = 0.9852$; [12]Significant linear regression: $y = 65.256 + 2.1347x$, $R^2 = 0.9982$.

IV. CONCLUSION

A higher incorporation of DDGS in the diet increased polyunsaturated fatty acids and raised the iodine value. This suggests that the belly fat oxidizes more quickly and becomes more flexible. Although this may reduce the bacon's slicability, it indicates a healthier fat profile for human consumption.

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LAIRAGE OVERNIGHT AT THE ABATTOIR – GOOD OR BAD?

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I. INTRODUCTION

Ongoing structural rationalizations have led to closure of Swedish abattoirs followed by prolonged journeys for slaughter livestock and a significantly increasing proportion of animals staying overnight at the abattoirs. In Sweden of today, about 25-70 % of the animals stand overnight at the medium sized and large abattoirs that slaughter a vast majority of the animals (Algers, pers. comm.). In the literature, some studies conclude that lairage time after transport potentially allows the animal to rest, renew muscle glycogen and reduce dehydration of body tissues and carcass weight loss [1]. Other authors suggest that the lairage environment itself may inhibit the ability of the animals to rest and recover from the feed and water restriction [2]. The discrepancies in these results indicate that more research is needed to further investigate the effects on slaughter animals under Swedish handling conditions. Hence, the aim of this study was to investigate whether lairage overnight at the abattoir lead to a reduction in animal welfare and meat quality compared to slaughter on the day of arrival.

II. MATERIALS AND METHODS

A total of 63 dairy steers were included. The aim was to send the animals to slaughter when they reached a live weight of 650 kg. As they varied in age, 24 animals went to slaughter before grazing while the remaining 39 went to slaughter after grazing in the autumn. However, the latter group was housed for at least four weeks before slaughter to ensure equal rumen filling. All animals were transported the same distance to the same abattoir. Once at the abattoir, half were slaughtered on the day of arrival and half the next day. In connection with bleeding, blood samples were taken for analysis of lactate, glucose and cortisol. Directly after slaughter, samples of *M. longissimus thoracis et lumborum* (LTL) were taken for measuring drip loss. Carcass pH and temperature were measured 24 h after slaughter. After cutting, samples of LTL were tenderized at 4°C for seven days and then stored at -28°C until analysis. Prior to analysis, the meat was thawed overnight at 4°C. Colour (L*, a*, b*), combined thawing and cooking loss and Warner-Bratlzer shear force (WBSF) were measured. Data were analysed with Proc Mixed in SAS [3].

III. RESULTS AND DISCUSSION

There was no difference in live weight between the groups, indicating that the groups were equivalent (Table 1). However, for the animals slaughtered in spring, the carcass weight was significantly lower for the overnight animals compared to the animals slaughtered on the day of arrival (Table 1). This led to a dressing % that was 1.2 % points lower for the overnight animals, which corresponds to about 8 kg based on the current live weights. However, a similar difference could not be seen for the animals slaughtered in the autumn (Table 1). A possible explanation for this may be that, despite higher live weight, they had a lower conformation than the animals slaughtered in the spring. They thus had a lower proportion of muscle, and a higher proportion of bone, on the carcass and consequently a lower proportion of the carcass that could potentially lose weight.

Overall, there was no difference in the concentration of lactate, glucose or cortisol between overnight animals and those slaughtered on the day of arrival (Table 1), suggesting that there was no difference in stress level between the animals that stayed overnight and those that did not. The concentrations of the stress parmeters were in parity with what has been seen in previous studies in connection with

slaughter [4]. Further, there was no difference in pH between the groups slaughtered in the spring, however, we could see a difference between the groups slaughtered in the autumn (Table 1) but both groups were within the desired range. We also could not see any difference in the colour of the meat between the groups (Table 1). The total fluid loss was the same for both groups slaughtered in the spring but higher for the overnight group among the animals slaughtered in the autumn. The higher fluid loss in the autumn overnight group may be due to physiological changes in protein structure. Despite no, or very small, differences in pH between the groups, we saw a significantly higher WBSF in meat from overnight animals regardless of whether they were slaughtered in spring or autumn. The fact that we saw such a large difference in tenderness despite no direct difference in pH is unexplained. A higher fluid loss also leads to a less tender meat, but the differences we saw here are not large enough to explain the large difference in tenderness.

Table 1 – Live weight, carcass characteristics and technological meat quality parameters of steers either slaughtered on the day of arrival or after one night in lairage.

	Spring slaughter			Autumn slaughter		
	No lairage	Lairage	Sign.	No lairage	Lairage	Sign.
n	*12*	*12*		*19*	*20*	
Live weight (kg)	644	635	ns	663	669	ns
Carcass weight (kg)	320	308	**	330	334	ns
Dressing (%)	49.6	48.4	*	49.8	49.9	ns
Confimation[2]	5.0	4.6	ns	4.3	4.1	ns
Fatness[3]	6.6	6.7	ns	7.4	7.3	ns
Lactate (mmol/L)	3.35	3.37	ns	3.74	4.11	ns
Glucose (mmol/L)	4.75	4.93	ns	4.02	4.88	***
Cortisol (nmol/L)	134	118	ns	128	111	ns
pH	5.76	5.61	ns	5.74	5.55	**
Drip loss (%)	1.86	1.26	**	1.75	1.60	ns
Lightness (L*)	26.2	25.6	ns	25.6	25.1	ns
Redness (a*)	14.2	14.1	ns	15.6	15.4	ns
Yellowness (b*)	14.1	14.6	ns	15.7	15.3	ns
Fluid loss (%)	26.5	26.6	ns	28.1	31.0	*
WBSF (N/cm^2)	59.7	91.2	**	44.5	76.6	***

IV. CONCLUSION

There were no differences in stress parameters between animals slaughtered on the day of arrival compared to those staying in lariage overnight. However, lairage led to a lower drip loss and significantly tougher meat.

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Exploring the impact of Halal and Jhatka slaughter on welfare, meat quality and proteomic changes in slow growing broiler chicken

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I. INTRODUCTION

Slaughtering is a critical step in the meat production process that affects animal welfare and meat quality. Different slaughter techniques may exert varying level of stress and impacts post-mortem muscle metabolism affecting meat quality. Current study explores the impact of two ritual slaughter techniques like halal and jhatka that are normally performed without any stunning prior to bleeding. Various stress parameters influencing welfare and meat quality were assessed in slow growing broilers.

II. MATERIALS AND METHODS

A total of 75 slow-growing broiler chickens (50-day-old males, Plymouth Rock x Red Cornish breeds, multi-coloured) were divided into three experimental groups (Jhatka slaughtered [JS], Halal slaughtered [HS], and slaughter with electrical stunning [ES]) in a completely randomised design. The experiment was replicated on five different occasions with 5 birds in each group (n=25). In JS and HS groups, birds were traditionally slaughtered without any stunning. After slaughter, blood and meat samples were collected and analysed for stress-indicating markers, including blood biochemical, enzymatic, and hormonal changes, meat quality and proteomic analysis. Statistical analysis using a two-way ANOVA was performed with OriginPro software to evaluate the impact of the three slaughter methods during post-mortem storage at 1, 4, 8, 12, and 24 h, considering repeated measures for welfare and meat quality and proteomics analysis. Least-square means were determined for significant F tests ($P<0.05$) and differentiated using least significant differences.

III. RESULTS AND DISCUSSION

The lactate dehydrogenase (LDH) level was markedly elevated ($P<0.05$) in ES group, whereas higher ($P<0.05$) levels of cortisol and triiodothyronine (T3) and lower ($P<0.05$) concentration of creatine kinase were observed in JS and HS birds, respectively. The LDH and CK were reported extensively as indicators of stress and their increased concentration in plasma reflect changes in tissue function or sign of cell injury [1]. The blood glucose level, creatinine, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and thyroxine (T4) were found to be non-significant ($P>0.05$) between the groups. Bleeding efficiency was lowest in ES relative to JS and HS groups. Halal slaughter presented the maximum bleeding compared to others, which might be due to the incidence of ventricular fibrillation and arrest of the heart function during application of electric shock (stunning) [3]. The stress induced in ES and JS may cause vasoconstriction limiting the blood flow compared to halal (non-stunned) birds [4].

Higher ($P<0.05$) pH was observed in JS meat, whereas higher *a** value and WHC was found in ES samples. Higher WHC might be due to the net charge effect [5]. Similar results were reported for electrically stunned and non-stunned chicken breast samples [6]. The shear force values were higher ($P<0.05$) in the HS samples; however, no difference was observed for TBARS, cooking loss %, and MFI % between the groups. The 2-dimensional gel electrophoresis (2-DE) (Fig. 1) coupled to MALDI-TOF MS of meat samples has identified a total of 94 protein spots, out of which 14, 10, and 42 spots exhibited significant differences ($P<0.05$) in normalized volume, intensity, and area, between HS, JS and ES samples, respectively. Proteins demonstrating positive correlation with stress, namely Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and L-lactate dehydrogenase (LDH)-A chain were overabundant in JS broilers (Fig. 1). These proteins operate synergistically to modulate anti-stress capabilities, respond to heat stress, regulate metabolic and inflammatory responses, manage stress responses, and control energy metabolism [7] as evident by bioinformatics and string analysis (Fig 2).

Table 1. Blood biochemical and physio-chemical properties of breast muscle of broiler chickens with different slaughter techniques

	JS	HS	ES	RSE	P-value
Blood biochemical parameters					
Glucose (mg/dl)	244[a]	239[a]	212[a]	0.670	0.096
Creatinine (mg/dl)	0.35[a]	0.40[a]	0.40[a]	0.732	0.125
Total protein (g/dl)	4.95[a]	5.25[a]	4.25[a]	0.808	0.115
Lactate dehydrogenase (LDH) (U/L)	766[ab]	596[b]	863[a]	0.880	<0.05
Creatine kinase (CK) (U/L)	4144[a]	1601[b]	6063[a]	0.847	<0.01
Aspartate Transferase (AST) (IU/L)	240[a]	219[a]	208[a]	0.764	0.302
Alanine aminotransferase (ALT) (IU/L)	18.6[a]	21.6[a]	13.7[a]	0.833	0.15
Cortisol (µg/dl)	0.21[a]	0.15[b]	0.14[b]	0.521	<0.01
Triiodothyronine (T3) (ng/dl)	2.10[a]	1.50[c]	1.88[b]	0.868	<.0.001
Thyroxine (T4) (µg/dl)	3.45[a]	3.56[a]	3.43[a]	0.923	0.315
Meat quality parameters					
Bleeding efficiency (%)	3.61[b]	4.52[a]	2.74[c]	0.812	<0.05
WHC (%)	32.1 [b]	30.8[c]	35.0[a]	0.660	<0.001
TBARS	0.04[a]	0.04[a]	0.04[a]	0.863	0.949
Cooked pH	6.28[a]	6.08[b]	5.94[b]	0.539	<0.01
Cooking loss (%)	29.2[a]	30.7[a]	30.3[a]	0.862	0.269
Shear force (N)	14.8[b]	19.3[a]	15.5[b]	0.859	<0.001
MFI (%)	28.4[a]	28.5[a]	28.6[a]	0.860	0.989

[a–c] Means without a common superscript were determined to be significantly different between slaughter methods.
JS- jhatka slaughter; HS – halal slaughter; ES – electrical stunning; RSE – residual standard error

Figure 1. Representative 2DE gel and MS spectra (GP3 & LDH) of Jhatka slaughtered chicken

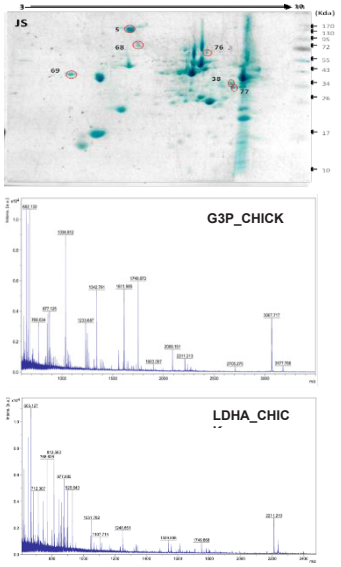

Figure 2. String analysis

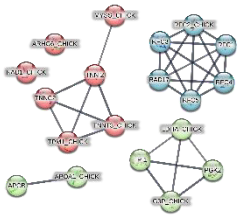

Table 2. Differential abundance in *Pectoralis major* muscles of jhatka slaughtered (JS) slow-growing broiler chickens identified through MALDI-TOF/MS analysis.

Spot ID[a]	Proteins	Accession no.	Spot ratio[b]
38	Glyceraldehyde-3-phosphate dehydrogenase	G3P_CHICK	4.21
77	L-lactate dehydrogenase A chain	LDHA_CHICK	3.13

IV. CONCLUSION

Current findings explore the impact of different slaughter techniques on meat quality as well as animal welfare. By comprehending the changes that occur during the slaughter process, producers can make well-informed decisions about ways to enhance meat production and animal welfare standards.

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IMPACT OF SPACE ALLOWANCE ON VEAL CALVES' BEHAVIOR AND PERFORMANCES

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I. INTRODUCTION

Veal production and its consumption are specific to France. Indeed, France is the world's leading consumer of veal calves with 3.2 kg carcass weight equivalent per capita. In 2022, approximately 1.2 million calves were slaughtered in France, which is the second largest producer in the world behind the Netherlands and ahead of Italy [1]. Veal calf production in France is organised around specialised fattening farms, mostly in closed facilities with dynamic ventilation. This type of production is organised per batch, with all calves (from dairy farms) arriving at the same time in the fattening facility at an average age of about 20 days. In most farms, they are housed in groups of 5 to 8 calves on wooden slatted floor. Throughout their fattening period, they are fed with milk and solid feed (a mixture of cereals, proteins and fibre) until they leave for the slaughterhouse (after about 5.5 months of fattening) [2]. In a context of structurally decreasing volumes produced and consumed, the veal industry must face up to new societal expectations and to a potential change in regulations concerning housing conditions, to improve the welfare of calves [3]. In 2021, the European Commission undertook to present a new legislative proposal on animal welfare by the end of 2023. To this end, the European Food Safety Authority (EFSA) has been asked to provide scientific advice on farm animal welfare. Although no measures have yet been adopted, EFSA's recommendations on veal calves, published in March 2023, provide an initial overview of the issues that could be discussed, such as an increase of calf space allowance from 1.8 m² to 3 m² per calf. In this context, a trial was carried out to investigate the impact of the space allowance in veal calves' production.

II. MATERIALS AND METHODS

At the Calf Research and Innovation Center (CIRVEAU), 83 male Prim Holstein calves were divided into three batches with three different space allowances per calf 1.8 m²(control), 2.25 m² and 3 m² per calf. The calves were initially housed individually for the first 28 days. After this period, they were grouped collectively. In one configuration, there were 5 calves per pen, and each pen had an area of 9m², resulting in a density of 1.8 m² per calf. In another setup, there were 4 calves per pen, with each pen providing 2.25 m² per calf. Lastly, in the 15 m² pen, 5 calves were housed together, allowing a density of 3 m² per calf. The calves were fattened for 168 days on wooden slatted floor and received the same feeding plan on a base of 250 kg of solid feed. Twice a day (at 7:15 am and 5:30 pm) and during the whole fattening period, reconstituted milk was distributed individually in buckets with feeding teats. The solid feed was then distributed in collective troughs. Water was available to the calves. The health protocol was identical for all calves (vaccination on arrival against RS-BVD and ringworm, as well as an anti-lice treatment). Calves were weighed every 28 days. At each weighing, a cleanliness score was given to each calf. Individual milk consumption was measured, as well as collective solid consumption (per pen). All sanitary treatments were recorded individually and daily. Regarding calves' activities as play behaviors or abnormal behaviors, scans sampling observations on a 5-minute time step were carried out from 6 am to 8 pm on 3 days, complemented by continuous sampling observations (D33, D99 and D161). Pedometers were placed on one of the back legs on 8 calves per batch for at least 2 weeks around the 3 days of observations. They were used to measure the lying/standing position and the number of steps taken by the animals. Significance

differences (P<0.05) among samples were determined by analysis of variance (ANOVA) using the Least Square Difference method of the General Linear Model procedure of R (R project 4.2.3).

III. RESULTS AND DISCUSSION

Among the 3 play behaviors observed, head-to-head was the most frequent, followed by running behavior and finally overlapping (p-value < 0.01). There was no difference in the frequency of head-to-head behavior in the finishing period (D112-D175) between the batches (5.3 times per calf for the control batch vs 4.8 times per calf for the 3 m² batch, NS). The racing behavior became less and less frequent during fattening and was very infrequent in the finishing period for all batches. No significant difference was observed between batches in the total frequency of play behavior per calf. Furthermore, the calves in the 3 m² batch did more steps than the calves in the other batches, which seems to be due to the size of the pen (15 m² instead of 9 m²) than to the space allowance itself (table 1). Regarding the abnormal behavior, material sucking (pica) was the most prevalent, followed by tongue playing and finally foreskin sucking (p-value < 0.01). No difference was observed in the average time spent per calf in pica. The increase of space allowance did not reduce the duration of negative behavior of the calves, nor did it have any impact on zootechnical performance (batch 1.8m² = 1250 g/d, batch 2.25m² = 1227 g/d and batch 3m² = 1245 g/d, NS) or carcass quality (table 2).

Table 1 – Calves activity per batch

Indicators	Fattening period	Batch 1.8m²		Batch 2.25m²		Batch 3m²		
		Mean	SEM	Mean	SEM	Mean	SEM	P-value
Number of steps	Start-up	302.6	62.2	325.2	76.0	363.9	89.7	0.21
	Growth	296.6^{ab}	51.0	285.2^{a}	72.4	370.2^{b}	47.7	0.04
	Finishing	261.9^{a}	49.8	281.7^{a}	72.8	391.6^{b}	116.3	0.001
	Total	287.8^{a}	55.7	304.3^{a}	74.0	375.2^{b}	86.9	0.006

Table 2 – Carcass Characteristics

Indicators	Batch 1.8m²		Batch 2.25m²		Batch 3m²		
	Mean	SEM	Mean	SEM	Mean	SEM	P-value
Carcass weight (kg)	140.0	10.9	138.4	11.9	139.6	12.8	0.87
Ratio output (%)	54.2	1.64	53.9	1.63	54.2	1.2	0.65
Conformation P (%)	50		33	-	50	-	-
Conformation O (%)	50	-	67	-	50	-	-

IV. CONCLUSION

In conclusion, reducing density does not increase the average duration of play, nor does it reduce the time spent by calves in abnormal behavior. Further research is needed to explore other means, such as enrichment of the environment and adaptation of the feed ration, to enable calves to express their natural behaviour more easily.

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THE POTENTIAL OF INDIGENOUS CHICKENS IN SMALL-SCALE FARMING FOR MEAT PRODUCTION IN SOUTH-AFRICA

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I. INTRODUCTION

Most of the peri urban communities in South Africa have sought to indigenous chicken production as a means of generating income for their households. Indigenous chicken production became popular due to low production input demand, and superior adaptability to harsh environmental conditions [1] The growing awareness of human health, nutrition, and animal welfare concerns has led to the development of specialty markets for organic food, hence indigenous chicken meat production [2]. Indigenous chicken breeds/ecotypes kept under minimal output production systems are starting to gain recognition in formal markets, however their potential in meat production is not well known. Farmers are still lacking skills in raising chickens to ensure good quality meat that has potential to enter formal markets. Meat quality and consistency are important in ensuring consumer satisfaction [3] if the product has to sell in the market. The objective of this study was to assess the potential of indigenous chicken meat production in Gauteng South Africa to be used as a baseline study to quantify and perform the meat quality audits of indigenous chickens.

II. MATERIALS AND METHODS

The baseline study was conducted in five municipalities of Gauteng, viz, City of Tshwane, Merafong, City of Johannesburg, MidVaal and West Rand. Interviews were carried out on the farm using questionnaires. For traceability, the coordinates where the survey was done were recorded. Data was collected from farmers that were identified by the extension officers from the Department of Agriculture and Rural Development in the different municipalities. Data was collected from small-scale chicken indigenous farmers. The questionnaires were focused on finding out the management practices, flock sizes, housing availability, feeding systems, health measures, marketing and main uses of indigenous chickens in the peri urban areas of Gauteng.

III. RESULTS AND DISCUSSION

The preliminary results is based from subset of farmers, both males (31%) and females (62%) with age range from 31- 40 (6%), 41-50 (25%), 51-70 (28%) over 70 (6%). The surveyed farmers consisted of 84% black and 15% coloured people. Ninety percent of these farmers own land. The results showed that farmers (84%) do value indigenous chickens and most (52%) of them practice extensive farming, where they let their chickens scavenge, however they provide yellow maize for supplementing the diet of the chickens. Farmers believe that yellow maize enables indigenous chickens to grow fast. The same system was reported by McAinsh et al. (4) for the chickens in Zimbabwe. Majority of farmers (46 %) keep chickens for meat production, while 25 % of the farmers keep them for incomes, few of the farmers (9%) keep chickens for cultural practices and for other reasons. The breed/ecotypes that are commonly kept by the farmers are local non-descriptive ecotypes that are either hatched and grown in the household or bought from neighbours. Farmers also keep Black Australorp, Rhode Island Red chicken, Playmouth, Rock chicken and Orpington chicken breeds. The interviewed farmers have a flock size of at least 50 chickens on average. Farmers showed fast growth and body size of the birds as the most important attributes for breeding selection for both hens and cocks and for marketing of

their chickens. The average live weight of the chickens recorded from the farmers, ranged from 1.2-2.3 kg. it is worth mentioning that animal age, breed, diet and production system were not considered when the taking the chickens weights, as it would be difficult to get the reliable information from the small-scale farmers. Majority of farmers do not keep such records. Most of the farmers use cage system to house their chickens, few of them use deep litter system or folding unit housing. Most of the houses are made with iron sheets with ground soil on the floor. While some farmers use wood, mud and bricks.

The above results showed that there can be potential for indigenous chicken farming in meat production, because majority of small-scale farmers own farming land, however this may be hampered by the challenges farmers are experiencing. The Major challenges facing the indigenous chicken small-scale farmers is the capital/financial input, followed by feed resource availability, disease and lack of veterinary care, skill in animal production and farm management, followed by skill in marketing strategies (most of the farmers sell their chickens from home to informal markets and cull chickens when they are too old >12months and use them for home consumption instead of selling them. Reveiwed studies in Manyelo et al. [5] have shown the same challenges in the production of indigeneous chickens in Africa. The challenges experienced by the farmers could be the reason of the small flock size. Most of the farmers (84%) do not belong to any of the farmers unions or producers organisations, which may be the major cause of not being able to overcome some of their challenges.

IV. CONCLUSION

The preliminary results revealed that the small-scale indigenous chicken farmers adapt their farming systems to the resources available. It is evident that indigenous chickens contribute to total production and consumption of poultry meat, but the actual level of contribution is difficult to estimate. Unless the challenges facing small scale indigenous chicken farmers are attended to, it will take a long way for indigenous chicken to reach optimal meat production to enter formal markets. In addition, low-input farming systems present risks for the commercial due to variability of the different properties that constitute to meat quality [6]. Therefore, there is a need for farmer education in raising indigenous chickens, especially for meat production. More farmer days should be held to raise awareness in indigenous chicken farming, how to access markets, record keeping, disease identification, biosecurity and reproduction and farm management.

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Durvillaea antarctica and agar powder new ingredients in the diet of meat lambs.

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I. INTRODUCTION

In the coming decades, the demand for animal protein will not be able to be covered by current production systems, so it is necessary to evaluate new strategies that involve greater intensification, but that are also sustainable and environmentally friendly [1]. In this context, the use of alternative feeds and materials to feed production animals is a focus of interest for the scientific community. *Durvillaea antarctica* is a brown seaweed that inhabits the southern hemisphere, which has recently been described and contains numerous nutritional elements [2]. Agar is a powder produced from red algae and is used to produce *in vitro* cultures of different plant species and microorganisms, but once used, it is disposed of as conventional waste. It has been reported that sheep in some island areas eat seaweed as a dietary supplement, without affecting their health or metabolism [3], so this study sought to evaluate the use of *Durvillaea antarctica* meal and agar in the diet of fattening lambs and its impact on the weight and overall quality of their carcasses.

II. MATERIALS AND METHODS

This study is endorsed by the Scientific Ethical Committee of the Universidad de La Frontera. Temuco, Chile. Two groups of lambs weighing 21.52 kg (n=20) and 15.65 kg (n=20), respectively, were housed and acclimatized for 15 days. Then, they were separated into modules. In the first module, one group (n=10) was selected as a control and a second group was fed 5% agar powder meal based on dry matter intake (n=10). In a second module, one group was selected as a control (n=10) and the other group was fed 5% *Durvillaea antarctica meal* (n=10) on a dry matter intake basis. The weight and health of the animals was monitored weekly. The design of the experiment was based on the work of Tripathi & Karim [4]. After 9 weeks the animals were slaughtered at the local slaughterhouse and the carcasses were taken to the laboratory in a 4°C refrigerated truck. The pH was measured 24 h *post mortem* on the *Longissimus* dorsi muscle *with* a pH meter by puncture (IQ150, IQ, Scientific Instruments, USA) and the color was measured with a colorimeter (CR-10 color-reader, Konica Minolta, Japan).

III. RESULTS AND DISCUSSION

The preliminary results of this study indicate that in general the animals fed 5% seaweed meal or agar powder did not present a different weight in relation to the respective control groups (Table 1). This study approached with caution the inclusion of both alternative raw materials since the literature suggests not to exceed 10% of inclusion of elements such as algae and by-products because they can generate alterations in the digestibility and health of the animals. Meat pH and color are key aspects highly related to meat quality and the 5% inclusion of *Durvillaea antarctica* and agar powder did not significantly affect these parameters, so we assume that these types of raw materials for feeding ruminants do not have negative effects on post-mortem carcass quality or meat quality traits, so they could be used as an alternative to feed of vegetable origin, which are increasingly scarce and whose production is not very environmentally friendly.

Table 1 – Effect of the inclusion of 5% agar powder and *Durvillaea antarctica* meal on live weight, pH and color of lamb meat.

	Treatment		
	Control ± SD	A. powder ± SD	p value
Live weight initial	16.22 ± 1.92	15.08 ± 1.90	0.05
Live weight final	18.66 ± 3.72	19.40 ± 3.57	0.73
pH	5.67 ± 0.09	5.70 ± 0.08	0.05
Color			
a*	18.32 ± 2.27	18.97 ± 2.26	0.49
b*	12.73 ± 1.50	14.17 ± 1.70	0.37
c*	22.34 ± 2.54	23.67 ± 2.60	0.49
L*	42.11 ± 2.65	45.31 ± 3.22	0.81

	Treatment		
	Control ± SD	*D. A* meal ± SD	p value
Live weight initial	20.22 ± 5.14	20.71 ± 4.35	0.05
Live weight final	29.33 ± 7.47	31.45 ± 5.96	0.48
pH*	5.57 ± 0.05	5.68 ± 0.09	0.00
Color*			
a*	13.89 ± 1.45	12.41 ± 1.86	0.05
b*	11.89 ± 0.63	10.77 ± 1.03	0.01
c*	18.29 ± 1.16	16.48 ± 1.74	0.03
L*	40.70 ± 3.31	41.18 ± 4.27	0.92

*The pH and color were measured on the *Longissimus* dorsi muscle. D.A meal: *Durvillaea antarctica* meal. The Mann-Whitney U test was used to evaluate differences between groups. Values are expressed as means ± standard deviation.

IV. CONCLUSION

The inclusion of agar powder and *Durvillaea antarctica* meal in the sheep diet does not seem to have negative effects on animal health or carcass quality. However, it is necessary to evaluate other aspects related to the physicochemical characteristics of the meat such as the fatty acid profile and proximal composition, which could have been modified due to the nutrients present mainly in the marine algae.

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EFFECTS OF ADDING A QUALITY COMPLEX ADDITIVE IN A STARTING DIET ON FEEDLOT PERFORMANCE OF BEEF CATTLE

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I. INTRODUCTION

Numerous feed additives can be used in the fattening diet of livestock aiming to improve feedlot growth performance parameters, such as weight gain, feed consumption, feed efficiency, mortality, morbidity, and carcass quality, which are all closely related to production costs and likelihood of profitability. Some examples of such feed technologies can be represented by acidifiers, essential oils, fatty acids, amino acids, probiotics, prebiotics, vitamins, minerals, enzymes, and phytogenics, among others [1,2]. A balanced combination of nutrients and specialty feeds has shown the ability to positively modulate intestinal tract microbiota activity while conferring immune system-stimulating properties [1]. Thus, the present study was designed to evaluate the effects of the dietary addition of QUALITY COMPLEX ADDITIVE® (QCA) offered during receiving diets on feedlot cattle growth performance.

II. MATERIALS AND METHODS

The experimental phase of this study was carried out in a feedlot located in Veracruz; a herd was offered treatments during the initial stage of production, as follows: A) nutritional packet QCA (0 and 3 kg/ton feed; DM basis) for 15 and 21 days, depending on the weight of the animal (heavy and light cattle, respectively); and B) Control (no nutritional packet). Nutritional packet QCA is mainly composed of yeasts, vitamins, and minerals. Three assessments [3,4] were performed: 1) mortality and morbidity were registered using 20 non-supplemented pens (Control, n = 1211 animals) and 20 QCA-supplemented pens (n = 1265 animals); 2) feed consumption was recorded for 25 days (Control, n = 1576 animals; QCA, n = 7335 animals); and 3) at the end of feeding cycle the average daily gain (ADG) and feed conversion (FC) were recorded (Control, n = 49173 animals; QCA, n = 29261 animals). The results were expressed as mean ± standard deviation and a t-test was used at $P < 0.05$ (NCSS version 2011).

III. RESULTS AND DISCUSSION

As shown in Figure 1, the inclusion of QCA in the initial diet positively affected feedlot growth performance and animal health. A notable decrease in mortality ($P < 0.001$) and disease incidence ($P < 0.001$) was observed among animals that received QCA compared to the Control group. In addition, a greater ($P < 0.001$) feed consumption and ADG ($P < 0.05$) were observed in the QCA group (from day 11 to day 25) and improved FC ($P < 0.001$). Current findings strongly suggest its potential application as an effective strategy to optimize cattle performance and health in intensive production systems. It has been reported that QCA supplementation in the finishing diet (last 64 days) improved the apparent total digestibility of nutrients in the tract, which increased the deposition of subcutaneous fat without affecting the growth performance of the animal and the quality of the carcass [5]. Moreover, it has been reported that the nutritional packet QCA is mainly composed of yeasts, vitamins, and minerals, and it is reported that these components seek to improve digestive health and counteract livestock challenges with energy diets. Live yeasts are known to stabilize pH and increase digestibility,

vitamins promote microbial growth and reduce oxidative stress, and are crucial for metabolism, while electrolytes are essential for homeostatic balance [5,6].

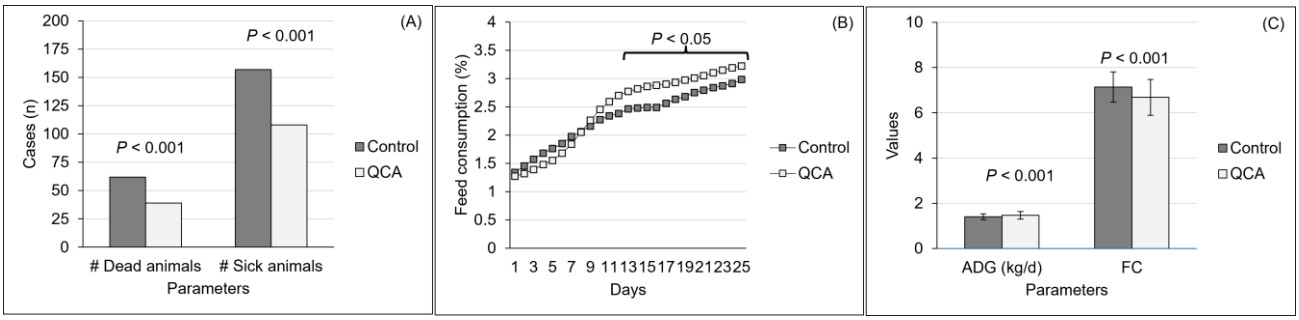

Figure 1. Effect of incorporating QCA into a starting diet on selected productive parameters of beef cattle. Average daily gain (ADG) and feed conversion ratio (FC).

IV. CONCLUSION

In summary, the results presented support the usefulness of QCA as a valuable supplement to improve beef cattle's productivity and welfare in the initial phase of intense fattening. However, additional studies are recommended to understand the underlying mechanisms of action better and thus be able to evaluate its long-term economic viability in different livestock production scenarios.

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THERMAL STRESS AND TRANSPORT DENSITY: INFLUENCES ON TILAPIA (OREOCHROMIS NILOTICUS) FILLET QUALITY

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I. INTRODUCTION

Pre-slaughter transportation is an important stage in the aquaculture process, where inadequate density can cause significant stress, directly affecting fillet quality. Studies indicate that stressful handling during transportation, including catching, loading, high density in tanks, and temperature fluctuations, provoke adaptive physiological responses in fish, influencing their health and well-being [1]. During periods of stress, energy supply is regulated, resulting in delayed rigor mortis, which is determinant for maintaining freshness and meat quality. Preventing rapid autolysis, which compromises the texture, juiciness, and shelf life of the product, is essential to ensure efficient processing and high-quality fillets [2]. Therefore, this study aimed to evaluate the effects of different densities in the pre-slaughter transportation of tilapia in winter and summer on fillet quality.

II. MATERIALS AND METHODS

One hundred eighty Premium® tilapia (*Oreochromis niloticus*) were used, with an average weight of 930 ± 150 g and an average length of 37 ± 3 cm. The fish were transported in 1000 L fiberglass tanks, equipped with diffusers and oxygen cylinders, for an average distance of 100 km over 1.5 hours. The experimental design was completely randomized in a 3 × 2 factorial scheme, with three densities (375, 425, and 475 kg/m³) and two seasons (summer and winter). After arriving at the slaughterhouse, the fish were stunned with benzocaine (1 g/10 mL of alcohol/10 L of water) and euthanized by spinal cord dissection, followed by evisceration and analysis of the left fillet. The pH was measured using a portable pH meter (Testo, model 205). Color was assessed at three different points on the fillet using a portable colorimeter (Minolta CR-10™) according to the CIE L*, a*, b* system. Water holding capacity was determined according to [3]. The data were tested for normality (Shapiro-Wilk, $p < 0.05$) and homogeneity of variances (Bartlett, $p < 0.05$), followed by analysis of variance and Tukey's test ($p < 0.05$), using STATISTICA® software.

III. RESULTS AND DISCUSSION

Fish transported at a density of 375 kg/m³ in winter showed the lowest pH value, followed by fish transported at 475 kg/m³, which did not differ from fish kept at 425 kg/m³, both also in winter (Figure 1a). The reduction in pH, indicative of greater *post-mortem* lactic acid production, results in greater acidification of the muscle. This phenomenon was exacerbated by thermal stress in winter, especially at lower densities, which do not adequately maintain the fish's body temperature [4]. Water holding capacity (WHC) also varied seasonally, being lowest for fish at 425 kg/m³ in winter, followed by densities of 375 kg/m³ and 475 kg/m³ (Figure 1b). The lower WHC associated with low pH is due to the denaturation of muscle proteins and greater exudation of water to the surface of the meat, which consequently explains the higher luminosity value (L*) (Table 1) found in the animals tested in winter [5]. Regarding the red (a*) and yellow (b*) intensity parameters, the highest values were observed in summer (Table 1), reflecting the greater production of pigments in response to stress [6].

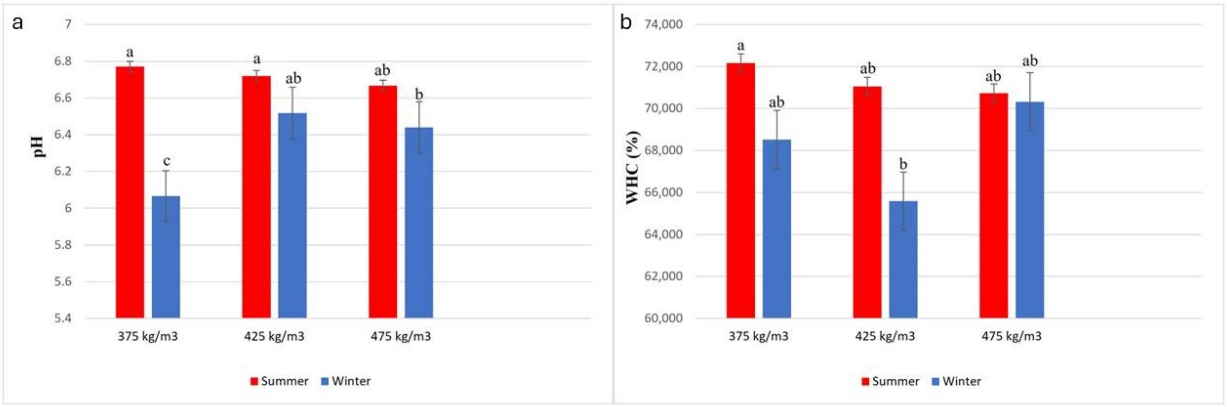

Figure 1. The bars represent the mean (± standard error) of the interaction between the variables (a) pH and (b) water retention capacity (WHC) of tilapia transported at different densities in summer and winter.

Table 1 - Mean values (± standard error) of the variables luminosity (L*), red/green hue (a*), and yellow/blue hue (b*) of tilapia transported at different densities in summer and winter.

Traits	Densities			Season		p-value		
	375 kg/m^3	425 kg/m^3	475 kg/m^3	Summer	Winter	Density	Season	DxW
L* _Surface	44.12±2.81	44.89±2.50	44.39±2.03	44.61±1.56^b	45.53±1.86^a	0.417	0.001	0.094
a*_Surface	5.56±1.59	5.77±1.94	5.33±1.53	7.05±0.88^a	4.62±1.20^b	0.581	0.000	0.649
b*_Surface	9.89±1.40	9.75±1.44	9.59±0.98	10.37±0.78^a	9.31±1.18^b	0.9742	0.029	0.596

IV. CONCLUSION

The combination of low transportation densities and lower temperatures exacerbates thermal stress, thereby compromising the final quality of the fillets. Optimal management of these factors is determinant to ensure high-quality tilapia products.

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CARCASS AND MEAT QUALITY TRAITS OF NELLORE YOUNG BULLS AND STEERS FED A HIGH OR LOW CONCENTRATE DIET THROUGHOUT FATTENING

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I. INTRODUCTION

Castration has been widely applied in beef production to improve meat quality characteristics. Compared to bulls, steers generally have higher carcass fat [1], higher marbling content [2], and increased meat postmortem tenderization rate [3]. On the other hand, under the same feeding conditions, bulls have higher feed efficiency and heavier carcasses than steers [4]. Therefore, the objective of this study was to evaluate the effect of castration on carcass and meat quality traits of Nellore bulls and steers fed a high or low-concentrate diet throughout fattening.

II. MATERIALS AND METHODS

A total of 28 young Nellore cattle, averaging 295.6 ± 8.05 kg of body weight and 8.0 ± 0.07 months of age, were used in this study. Half of the calves were randomly selected for surgical castration one week after weaning. Post-weaning, calves were confined for rearing and subsequently adapted to two finishing diets with different roughage:concentrate ratios: 50:50 and 15:85. Therefore, four experimental groups were generated: Steers 50:50, N=7; Steers 15:85, N=7; Bulls 50:50, N=7; and Bulls 15:85, N=7. The animals were slaughtered after 122 days of feedlot trial, with an average weight of 454.4 ± 30.0 kg. After harvest, data on hot carcass weight (HCW) and cold carcass weight (CCW) were obtained. After 24 hours of chilling, *Longissimus lumborum* (LL) samples were collected for meat quality analysis. The subcutaneous fat thickness (SFT) was measured in the *Longissimus lumborum* (LL) muscle using a digital caliper, and the Warner-Bratzler shear force (WBSF) was determined following American Meat Science Association guidelines [5]. Sarcomere length was estimated according to the laser diffraction technique [6]. The myofibrillar fragmentation index (MFI) was assessed by measuring the turbidity of homogenized samples in a standardized protein concentration [7]. All data were analyzed as a completely randomized design following a 2x2 factorial arrangement of treatments (2 sex conditions and 2 roughage to concentrate ratios). Analysis of variance (ANOVA) was performed to evaluate the effect of main factors and interaction on carcass and meat traits, using the GLM procedure of SAS. Once detected significant effect ($P \leq 0.05$) for sex at diet or interaction, treatments were compared by Tukey's test. Also, tendency was assumed when $0.05 < P \leq 0.10$.

III. RESULTS AND DISCUSSION

There was a strong tendency for sex variation in hot carcass weight (HCW) ($P = 0.064$) and cold carcass weight (CCW) ($P = 0.069$). Interaction between sex and diet was observed for HCW ($P = 0.023$) and CCW ($P = 0.024$). The pH of the bull group tended to be higher than the pH of the steer group ($P = 0.082$). A strong tendency towards a sex x diet interaction was observed for meat pH ($P = 0.058$). The WBSF was not affected by diet and sex ($P > 0.05$). On the other hand, there was a difference in SFT ($P = 0.048$) and MFI ($P = 0.041$) among sex treatments. Steer carcasses showed higher SFT than bull carcasses similar results were previously reported [8]. These results suggest that when castrated at weaning and fed in an intensive feeding system, Nellore steers show greater fat deposition. The higher MFI of steers can be explained by increased lipid uptake and lipogenesis, and decreased lipolysis, compared to bulls [9]. Tenderness is considered one of the most important characteristics of meat quality, but it is also highly variable [10].

Table 1 – Carcass and meat quality traits of Nellore young bulls and steers fed a high or low concentrate diet throughout fattening.

	Steers		Bulls			*P- value*		
	50:50	15:85	50:50	15:85	SEM	Sex	Diet	Sex*Diet
HCW, kg	126.43^{A}	128.01^{A}	133.29^{A}	138.81^{B}	2.80	0.064	0.398	0.023
CCW, kg	124.24^{A}	125.96^{A}	130.96^{A}	136.43^{B}	2.74	0.069	0.398	0.024
Sarcomere, µm	1.40^{A}	1.30^{A}	1.30^{A}	1.31^{A}	0.02	0.875	0.090	0.100
SFT, mm	7.99^{A}	6.90^{A}	6.42^{A}	5.65^{B}	0.49	0.048	0.076	0.455
WBSF, N	27,68^{A}	30.60^{A}	30.30^{A}	29.53^{A}	0.65	0.228	0.284	0.883
MFI, %	39.66^{A}	30.08^{AB}	28.11^{B}	31.41^{AB}	2.54	0.041	0.067	0.577

SEM: Standard error of the mean; For each variable, within a row, means without a common superscript letter are significantly different. Significant differences at 5% probability (P⩽0.05). Tendency was assumed when 0.05 < P ≤0.10. HCW: Hot carcass weight (kg); CCW: Cold carcass weight (kg); SFT: Subcutaneous fat thickness (mm); WBSF: Warner-Bratzler Shear Force (V); S: Sarcomere (µm) MFI: Myofibrillar fragmentation index (%).

IV. CONCLUSION

This study indicates that castration improved carcass quality regardless of diet during fattening by improving fat deposition. However, it did not influence meat tenderness.

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EVIDENCE OF INTERACTION BETWEEN THE DEGREE OF DAILY FLUCTUATION IN DRY MATTER INTAKE AND PRODUCTION TRAITS IN NELLORE CATTLE

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I. INTRODUCTION

The degree of daily fluctuation in dry matter intake (DMI) in Nellore cattle was significantly related to the blood gas profile and rumenitis scores, indicating that high-DMI fluctuation had adverse effects on growth performance potentially associated with an increased incidence of metabolic disorders [1]. The present study seeks to determine whether daily fluctuation in DMI can influence the performance and carcass traits of Nellore cattle from the genetic improvement program for Nellore breeds at the Institute of Animal Science, Sertãozinho/SP, Brazil (a program designed to increase animals' post-weaning weight and whose selection is carried out based on individual performance).

II. MATERIALS AND METHODS

For this study, 107 non-castrated 15-month-old Nellore males (358 ± 5.56 kg of live weight) were housed in experimental pens, fed high-concentrate diets for a minimum of 92 days, following the distribution of a completely randomized design, in 2 x 3 factorial arrangement: two groups of DMI fluctuation (GF of DMI: high and low fluctuation) and three genetic groups (GG: control Nellore-NeC, selection Nellore-NeS and traditional Nellore-NeT). The fluctuation was calculated by the difference between DMI of the previous and the current day [%F-DMI = $((DMI_{Previous} - DMI_{Current}) *100)/ DMI_{Previous}$], expressed as percentage. Then, animals were classified as high (H: 2.01%) or low (L: 0.90%) DMI fluctuation (P=0.001). Genetic groups of Nelore cattle were established based on selection for higher yearling body weight (NeS and NeT), or selection for mean yearling body weight (NeC). NeS and NeC were closed since the beggining of the program, while NeT eventually received bulls from other herds [2]. The criteria for slaughter was when animals had reached the minimum of 4 mm of subcutaneous fat thickness over the 12th rib measured by ultrasound. The performance and carcass traits were analyzed by PROC MIXED of SAS, considering the fixed effects (GF and GG), the covariate (age at slaughter), and the random effect (year). The differences between means were compared using the Tukey test ($P<0.05$), and trends were verified when $P<0.10$.

III. RESULTS AND DISCUSSION

The degree of DMI fluctuation did not influence ADG, DMI, FER, RFI, pH, CY, luminosity (L*) and yellow content (b*) means ($P>0.05$). However, it was observed that animals from high-DMI fluctuation had lower FBW compared to the ones from low-DMI fluctuation group (P=0.001). Likewise, there was a tendency towards greater HCW, CCW, BFT, and REA to cattle from low-DMI fluctuation group when compared to cattle from the high-DMI fluctuation one.

For GG results, selection for growth was highly effective in increasing body and carcass weights, what can be seen comparing NeT and NeS groups with NeC. Selected animals had greater live body weights, hot and chilled carcass weights ($P<0.05$) than control animals. However, these differences in body size did not influence FER, RFI, or meat quality traits ($P>0.05$).

Significative interactions between GF and GG were detected for DMI fluctuation, DMI and a*. The GF results show that within each GG, there were cattle with high and low DMI fluctuations. For DMI, there

was greater feed intake from low-fluctuation NeS animals compared to high-fluctuation NeS cattle. In the results of a*, NeC animals from the low DMI fluctuation group showed higher intensities of red color in the meat than NeT animals.

Table 1. Average productive traits and carcass traits of male Nellore cattle

[3]Parameter	[2]GF		[2]GG			[1]P Value		
	Low	High	NeC	NeS	NeT	GF	GG	GFxGG
DMI fluctuation, %	0.90	2.01	1.52	1.52	1.31	0.001	0.421	0.025
IBW, kg	345	337	316b	351a	356a	0.338	0.014	0.205
FBW, kg	487a	466b	432b	501a	497a	0.072	0.001	0.528
DMI, kg/d	9.50	9.44	8.92	10.04	9.46	0.083	0.063	0.039
ADG, kg/d	1.49	1.47	1.32b	1.60a	1.52ab	0.643	0.019	0.162
FER, kg/kg	0.16	0.16	0.16	0.17	0.17	0.369	0.161	0.119
RFI, kg/d	0.040	0.039	0.308	-0.109	-0.079	0.793	0.308	0.545
BFT, mm	4.82a	4.21b	4.35	4.52	4.67	0.085	0.906	0.393
REA, cm²	77.15a	73.50b	72.01	74.72	79.26	0.093	0.165	0.317
HCW, kg	285a	272b	250b	292a	295a	0.074	0.002	0.677
CCW, kg	281a	268b	246b	287a	290a	0.054	0.002	0.592
L*	30.53	30.84	29.35	31.70	31.01	0.615	0.215	0.090
a*	18.55	16.55	19.38	17.21	16.05	0.012	0.110	0.028
b*	8.41a	7.03b	8.36	7.79	7.01	0.089	0.598	0.118
pH	5.50	5.55	5.55	5.45	5.58	0.989	0.791	0.161
CY, %	58.34	58.15	57.79	57.93	59.00	0.615	0.262	0.733

[4]Significant interactions

GG	Flutuação CMS, %		P Value	CMS, kg/d		P Value	a*		P Value
	GF			GF			GF		
	Low	High		Low	High		Low	High	
NeC	0.98Ab	2.07Aa		9.00Aa	8.83Aa		20.89Aa	17.86Aa	0.028
NeS	0.82Ab	2.23Aa	0.025	10.21Aa	9.86Ab	0.039	18.89ABa	15.53Aa	
NeT	0.89Ab	1.72Aa		9.30Aa	9.62Aa		15.86Ba	16.23Aa	

[1]Means followed by the same capital letters do not differ in the columns, and same lowercase letters do not differ in the rows by Tukey test (P<0.05 or trend P<0.10); [2]GF: DMI fluctuation groups; GG: genetic groups (NeC: control Nellore; NeS: selection Nellore; NeT: traditional Nellore); [3]Dry matter intake, DMI; Average daily gain, ADG; Initial body weight, IBW; Final body weight, FBW; Feed efficiency ratio, FER; Residual feed intake, RFI; Rib eye area, REA; Backfat thickness, BFT; Hot carcass weight, HCW; chilled carcass weight, CCW; color (CIELAB system: L, a*, and b*);pH; Carcass yield, CY.[4]Significant interaction between factors (GF and GG)

IV. CONCLUSION

The fluctuation of DMI can interact mainly with live body weight and carcass traits of Nellore cattle, as well as influencing color attributes of the meat.
It is desirable to optimize monitoring and control processes of the production system to reduce variability in animal DMI, avoiding metabolic disorders related to performance in Nellore cattle.

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MEASUREMENT OF HUMAN AND ANIMAL RELATIONSHIP IN A SLAUGHTERHOUSE OF FATTENING PIGS USING CO_2 FOR STUNNING

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I. INTRODUCTION

Pig welfare and meat quality are important factors in the pig industry as they have a direct impact on production costs. Over the past few years, an increased focus on evaluating animal welfare in slaughterhouses [1]. Human-animal contact during transport and slaughter procedures for fattening pigs is crucial for the emotional state and appropriate behavior [2]. After the pigs leave the lairage, they are moved into the stunning chamber. This process involves an interaction between the animals and humans which can be evaluated by vocalizations. It has been hypothesized that high-pitched vocalizations (HPV) emitted by pigs in slaughterhouses can serve as indicators of the underlying animal and human relationship and the stunning method applied. High-pitched vocalizations emitted by pigs in slaughterhouses can provide insight into the nature of the human-animal relationship. This study consisted in the evaluation of the human-animal relationship through the record of vocalizations emitted by pigs when being moved from the lairage to the stunning area.

II. MATERIALS AND METHODS

Investigators recorded whether any of the observed group of animals vocalized during each 20-second interval (focal sampling) and whether any pig vocalized just at the end of the 20-second interval (scan sampling). The 20-second evaluation periods and focal scanning were carried out during locomotion 12 times per batch. The observations were conducted three times a day to assess three different farm batches in a total of 31 observation days. Vocalizations are presented as the number of times HPV occurred during the first four observation periods (HPV1_4), the second four observation periods (HPV5_8) and the third four observation periods (HPV9_12) concerning 1116 periods of 20 s that comprise the assessment. Spearman's correlation coefficients (P <0.01) were performed to study the relationships between variables.

III. RESULTS AND DISCUSSION

Table 1 presents correlation coefficients (P <0.01) and correspondent r values for the first four observation periods (HPV1_4), the second four observation periods (HPV5_8), and the third four observation periods (HPV9_12), one vocalization at the end of the 20-second interval, multiple vocalizations at the end of the 20-second interval and absence of HPVvariables studied.

Table 1 – Correlation coefficients (P <0.01) and correspondent r values for variables.

Variables		HPV1_4	HPV5_8	HPV9_12	One Vocaliz.	Mult. Vocaliz.	Absence HPV
				r			
HPV1_4		--	0.259	0.089	**0.281**	0.011	**-0.274**
HPV5_8	*P value*	0.012	--	0.184	**0.351**	**0.275**	**-0.392**
HPV9_12		0.395	0.077	--	0.075	0.091	-0.093
One Vocaliz.		**0.006**	**0.000**	0.474	--	0.140	**-0.957**
Mult. Vocaliz.		0.913	**0.008**	0.385	0.182	--	**-0.378**
Absence HPV		**0.008**	**0.000**	0.374	**0.000**	**0.000**	--

Significant correlations (P <0.01) and correspondent r values are presented with bold letter.

A significant positive correlation between HPV1_4 and and One_Vocalization (r = 0.281, p = 0.006), and between HPV5_8 and One_Vocalization (r = 0.351, p < 0.001) indicated that higher occurrence of HPV is also associated with occurrence of stantaneous vocalization in the first and second periods of locomotion for lairage to stunning indication a more stressful phase. Additionally, a significant positive correlation was observed between HPV5_8 and Mult_Vocalization, further indicating that higher levels of HPV5_8 are associated with increased instances of Mult_Vocalization. Furthermore, there is a significant negative correlation between Non_HPV and HPV1_4, suggesting an inverse relationship between Non_HPV and the HPV1_4 variable. Non_HPV also exhibits strong negative correlations with both HPV5_8 and Mult_Vocalization, indicating a considerable inverse relationship between Non_HPV and these variables. According to Dalmau et al. [3] there is a relationship between the stunning system and vocalization parameters (HPV during 20-second interval and vocalization at the end of the 20-second interval). In some slaughterhouses, as the one of this experiment which used CO2, animals are moved to the stunning area with automatic doors that reduce the human-animal interaction and thereby HPV (Figure 1). Actually, in the present study,the occurence of HPV (Figure 1) was low and the vocalization asssessement before the stunnig (9_12) was also the lower. Other studies also confirmed an association between vocalisations and the stunning system when assessed just before the stunning. However, , results can depend more on the way the animals enter the system than on the system itself (i.e. use of automatic doors vs use of an electric prod) [3, 4].

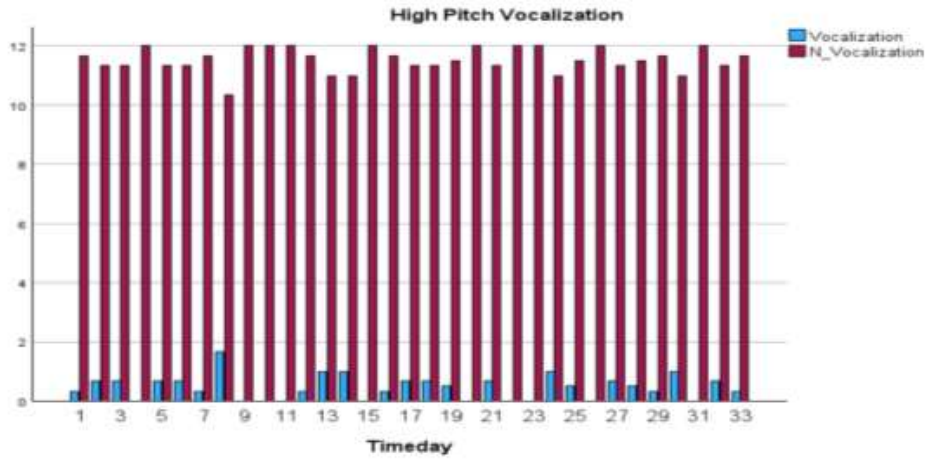

Figure 1. Number of HPV per observation day.

IV. CONCLUSION

This study evaluated the human-animal relationship through the record of vocalizations. Higher counts of HPV are associated with more focal scanning vocalization, indicating a more stressful phase. Additionally, the use of automatic doors to move animals to the stunning area seems to reduce human-animal interaction. The present study also found a low occurrence of HPV and lower vocalization assessments before stunning.

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CASTRATION METHOD AND TYPE OF WEANING INTERFERE IN THE PRODUCTIVE CHARACTERISTICS OF CONFINED ANGUS X NELLORE CROSSBREED BOVINE

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I. INTRODUCTION

Castration is a procedure that alters hormonal effects on metabolic pathways and the growth development of livestock animals, affecting meat quality attributes [1]. In Brazil, surgical castration is the most common method, but this practice can present post-surgical complications that cause stress and slow recovery. In this way, castration by rubber ring becomes a less invasive method that consists of fixing a high-pressure elastic ring around the base of the scrotum to suspend blood circulation until the testicles atrophy and are eliminated [2]. According to Coetzee [3], castration with a rubber ring leads to lower concentrations of blood stress indicators (35% lower cortisol) when compared with conventional surgical castration. Regarding weaning, this procedure is commonly performed in Brazil when calves reach between 7 and 9 months of age. However, early weaning carried out with animals aged 3 to 5 months has been gaining ground in livestock farming due to the development of the rumen caused by dietary changes, allowing for faster animal growth and a shorter production cycle. Therefore, the aim is to test whether early weaning or castration with a rubber ring benefits the performance and carcass characteristics of Angus x Nellore crossbred cattle finished in feedlots.

II. MATERIALS AND METHODS

A total of 24 Angus x Nellore crossbred cattle aged 12 months and live weighing 425 ± 6.93 kg were confined for 125 days following a completely randomized design in 2 x 3 factorial arrangement: two castration methods (surgical and rubber-ring) and two types of weaning (early and traditional), totaling 4 treatments with 3 replications of 2 animals each. The animals were kept under the same management conditions, receiving the same diet *ad libitum*. The performance analysis considered dry matter intake (DMI, kg/d), initial and final body weight (IBW and FBW, kg), average daily gain (ADG, kg) and feed efficiency (FER) as the ratio between ADG and DMI. The carcass traits evaluated considered hot carcass weight (HCW, kg), carcass yield (CY, %), pH, rib eye area (REA, cm²), backfat thickness (BFT, mm) and marbling score. The results were analyzed using PROC MIXED in SAS, considering the fixed effect (castration methods and type of weaning). The means of the results were compared using the *F* test, and the effects were considered significant when $P \leq 0.05$ and a tendency when $0.05 \leq P \leq 0.10$.

III. RESULTS AND DISCUSSION

The results of the performance and carcass characteristics of Angus x Nellore crossbred male cattle are shown in Table 1. The treatments did not affect IBW, FBW, HCW, and marbling ($P>0.05$). It was observed that animals castrated with a rubber-ring were 13.3% more efficient (higher FER, $P=0.0357$), increased ADG by 0.29 kg/d ($P=0.0109$), and CY by 2.3% ($P=0.0328$) compared with surgically castrated animals. There was a lower pH in males castrated with a rubber-ring compared with the pH values of surgically castrated cattle ($P=0.0002$). Similarly, there was a tendency towards lower REA and DMI (difference of 0.88 kg/d) between animals castrated with a rubber-ring and

those castrated surgically (P=0.0702; P=0.0918). Lastly, there was a significant tendency for DMI and BFT (P=0.0523; P=0.0794), in which early-weaned cattle had lower BFT and a 0.94 kg/d decrease in DMI compared with cattle from traditional weaning.

Table 1. Mean performance and carcass characteristics of Angus x Nellore crossbred cattle

[2]Variable	Castration (C)		Weaning (W)		[1]P-Value		
	Surgical	Rubber-ring	Early	Traditional	C	W	W x C
IBW, kg	436.00	409.00	433.67	411.33	0.1743	0.2542	0.3441
FBW, kg	558.03	555.37	562.06	551.35	0.9140	0.6450	0.5932
ADG, kg	1.14^{b}	1.43^{a}	1.25	1.32	0.0109	0.3994	0.1013
DMI, kg/d	8.86	9.74	8.83	9.77	0.0918	0.0523	0.2957
FER	0.13^{b}	0.15^{a}	0.14	0.13	0.0357	0.3082	0.1665
CY, %	56.45^{b}	57.76^{a}	56.99	57.22	0.0328	0.6355	0.3739
HCW, kg	317.53	318.22	322.61	313.13	0.9671	0.5629	0.6999
Marbling	511.95	535.73	504.04	543.64	0.7213	0.5285	0.4864
pH	5.59^{a}	5.50^{b}	5.55	5.54	0.0002	0.3829	0.3609
BFT, mm	12.02	12.14	10.58^{B}	13.58^{A}	0.9452	0.0794	0.6518
REA, cm²	74.18^{b}	81.67^{a}	77.61	78.24	0.0702	0.8606	0.1667

[1]Means followed by the same lowercase letters in each row and factor does not differ by F test (P<0.05 or trend 0,05 ≤ P ≤ 0,10). [2]Initial body weight, IBW; Final body weight, FBW; Average daily gain, ADG; Dry matter intake, DMI; Feed efficiency ratio, FER; Carcass yield, CY; Hot carcass weight, HCW; Backfat thickness, BFT; Rib eye area, REA; pH.

IV. CONCLUSION

Early weaning does not affect the cattle's productive traits and has brought small benefits regarding DMI and BFT. Cattle (F1 Angus x Nellore) castrated with an elastic ring showed slight benefits in productive characteristics such as ADG, FER, CY, and REA, indicating that it could be an alternative procedure for use in Brazil's cattle production system. However, more studies should be carried out to verify the effectiveness of the castration technique by increasing the number of animals tested.

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SESSION 4

Genetics and Physiology

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PROTEOMIC BIOMARKERS OF BEEF TENDERNESS FROM STEERS FED SORGHUM AS A SUBSTITUTE FOR MAIZE

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I. INTRODUCTION

Beef tenderness is determined during eating time and more tender meat results in repetitive purchases [1]. Dietary bioactive phytochemicals such as polyphenolic compounds can potentially be assimilated into the meat [2]. These compounds subsequently retard/inhibit enzyme activities involved in glycogen metabolism [3] and myofibrillar protein degradation thereby altering meat tenderness post-mortem [4]. Sorghum contains up to 30 g/kg dry matter (DM) tannins and it has been widely utilized in beef feedlot finisher diets to substitute maize, a more expensive energy source [5]. Studies have reported a reduction in tenderness when beef is finished with sorghum-based diets and attributed the changes to the effects of tannins on myofibrillar degradation and fat deposition [2]. However, little is known about the biochemistry of underlying processes and the major proteins involved in meat tenderness changes when finisher diets containing sorghum are fed. Thus, the current study objective was to identify proteins and biochemical pathways associated with beef tenderness from Angus steers fed graded levels of sorghum-based finisher diets.

II. MATERIALS AND METHODS

Twenty-one Angus steers were finished (90 days) with diets containing diets containing either 0 (SGD-0), 200 (SGD-200), or 400 (SGD-400) g/kg DM of sorghum grain substituting white maize grain. After 24 h post-mortem, the pH was measured with a portable handheld pH meter from the *longissimus thoracis et lumborum* (LTL) muscle which was harvested from the 9th to 13th rib, tenderness (Warner-Bratzler shear force; WBSF) determined, two-gram cubes were sampled from three loins per treatment and proteins were quantified with Bradford assay, visualized using SDS-PAGE and identified with LC-MS/MS [6]. To categorize the proteins intrinsic in beef tenderness, the identified protein gene names were compared with published literature data for tenderness [1]. The physical attributes data were analyzed using the GLIMMIX procedure of SAS.

III. RESULTS AND DISCUSSION

The inclusion of sorghum in beef finisher diets showed a linear increase ($P < 0.05$) in WBSF values which could be linked to a similar trend in dietary tannins that inhibit glycogen phosphorylase, lactate dehydrogenase and calpains thus limiting myofibril degradation [3,4]. Of the 11 differentially expressed proteins (FDR < 0.05), sorghum diets downregulated ($P < 0.05$) MYH1, MYH8, GYS1, HSPA8, HSP90AA1 and HSPB6 while CAPZB was upregulated ($P < 0.05$). The down-regulation of heavy chain myosin (MYH1 and MYH8) in SGD could be ascribed to the over-expression of CAPZB which provides binding sites for μ-calpain that breaks down myosin chains. The CAPZB over-expression is in turn attributable to the low calcium content of sorghum that reduces the abundance of phosphatidylinositol (4,5) bisphosphate that inhibits CAPZB activity. The downregulation of glycolytic proteins (GYS1 and PYGM) and heat shock proteins (Hsp) in sorghum diets is ascribable to the inhibitory effect of tannins [3]. Two (MYL3 and YWHAE) and 3 (HSPA9, PDIA3 and ANKRD2) tenderness-regulating proteins were uniquely expressed in SGD-200 and SGD-400, respectively. Several differentially and uniquely expressed structural proteins and glycolytic enzymes suggested that SGD could produce less tender beef which corresponded with instrumental tenderness results.

Table 1: Differentially regulated and unique proteins associated with tenderness in LTL from beef fed graded levels of sorghum

		Inclusion level (g/kg DM)			
Physical parameter		0	200	400	SEM
pH		5.7	5.9	5.8	0.04
WBSF		55.8^{b}	60.6^{ab}	65.2^{a}	2.18
Gene names	Protein names				
Differentially expressed					Mass (kDa)
MYH1	Myosin heavy chain 1	Up	Down	Down	220.8
MYH8	Myosin-8	Up	Down	Down	222.8
CAPZB	F-actin-capping protein subunit beta	Down	Down	Up	32.2
GYS1	Glycogen synthase	Up	Down	Down	76.5
PEBP1	Phosphatidylethanolamine-binding protein 1	Down	Up	Up	21.0
PYGM	Alpha-1,4 glucan phosphorylase	Up	Up	Down	97.3
PYGM	Glycogen phosphorylase, (Myophosphorylase)	Up	Up	Down	97.3
PARP6	Polymerase & Pyruvate kinase	Up	Up	Down	102.5
HSPA8	Heat shock protein family A (Hsp70) member 8	Up	Down	Down	72.3
HSP90AA1	Heat shock protein 90 alpha family class A member 1	Up	Down	Down	89.1
HSPB6	Heat shock protein	Up	Down	Down	17.5
Uniquely expressed					
RABGGTA	Geranylgeranyl transferase type-2 subunit alpha	Unique			64.9
HSPA5	Heat shock protein 70 family protein 5	Unique			72.4
APOBEC2	mRNA cytosine deaminase 2	Unique			26.0
MYL3	Myosin light chain 3		Unique		27.2
YWHAE	14-3-3 protein epsilon (14-3-3E)		Unique		29.2
HSPA9	Heat shock 70 kDa protein 9			Unique	71.2
PDIA3	protein disulfide-isomerase			Unique	51.9
ANKRD2	Ankyrin repeat domain 2			Unique	39.3

WBSF: Warner-Bratzler shear force; SEM: Standard error of means; Means within a row with different superscripts ($^{a–b}$) are different ($P < 0.05$).

IV. CONCLUSIONS

Overall, differentially and uniquely expressed proteins suggested that diets containing sorghum up to 400 g/kg yield beef of less desirable tenderness.

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Comparative analysis of pig lineages: impact on pre-slaughter weight and carcass traits

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I. INTRODUCTION

The implementation of genetic improvement programs has become essential to effectively combine the yield of carcass and meat quality in pig production (MIAR et al., 2014). One of the strategies that can be applied in genetic programs to enhance desirable traits in the offspring to introduce the genetic diversity through crossbreeding systems (KIM et al., 2020). Male pigs from specific lineages can be utilized to introduce beneficial genetic variation into the breeding population, leading to improved economic and quality traits in production. Crossbreeding can help produce pigs that are better suited to local environments, which can indirectly influence pork quality by promoting healthier, more resilient animals. Different pig breeds may have varying degrees of adaptation to specific environmental conditions such as climate, feed availability, or disease resistance (WILLSON et al., 2020). In addition, crossbreeding can help produce pigs that are better suited to stress conditionslocal environments, which can indirectly influence pork quality by promoting healthier, more resilient animals. Based on this, the main goal of this study was to evaluate the carcass traits of finishing pigs from three different terminal sire lines, such as Duroc, Hybrid (Duroc x Pietrain), and Pietrain.

II. MATERIALS AND METHODS

The experimental procedure on animal care and use was approved by Ethics Committee on the Use of Animals of the Escola Superior de Agricultura Luiz de Queiroz (University of São Paulo, Piracicaba, Brazil), CEUA nº 7416051222. A total of 600 DanBred Hybrid (DB90) males and female piglets sired by three different terminal lines (Duroc; Duroc x Pietrain (Hybrid); Pietrain) were allocated in 12 different pens with 25 pigs in each (four pens for each terminal sire line). All pigs were raised in the same management and nutritional system in the same fattening pig farm. At 169-day-raising period, the pigs were slaughtered, and the yield and quality carcass traits were evaluated on the left half carcasses. The initial and final body, cold and hot carcass weight, loin depth, and backfat thickness were measured and the hot carcass yield was calculated. To assess the effects of different crossbreeding systems on pork quality traits, we conducted an analysis of variance (ANOVA) with terminal sire line and housing age as a fixed effect and covariate, respectively, followed by Tukey test. All statistical analyses were performed using the R program, and significance was assessed at p-value ≤ 0.05.

III. RESULTS AND DISCUSSION

The initial and final body weight, yield and quality carcass traits are shown in Table 1. In this study, there was no difference ($p > 0.05$) in initial and final body weight among the terminal sire lines groups. The hot carcass weight (kg) was statistically different between the Hybrid and Pietrain groups (p=0.03), on the other hand, the hot carcass yield (%) was statistically different between Duroc and Pietrain and Duroc and Hybrid (p=0.002). The cold carcass weight (kg) and loin depth (mm) were not statistically different between Duroc and Hybrid groups, however, they showed a lower value compared with Pietrain group (p=0.018 and p=0.0139, respectively). The Pietrain group showed a lower cold carcass weight (kg), loin depth (mm), and higher backfat thickness (mm) compared with the other two groups. Herein, we observed that the applied crossbreeding strategy allowed the improvement in traits such

as hot and cold carcass weight, loin depth and backfat thickness for the Hybrid group. In other words, the Hybrid group showed higher muscle deposition and, consequently lower fat deposition when compared with the Pietrain group. These results are consistent with previous studies that highlighted the benefits of crossbreeding for improving productivity and carcass quality (KOWALSKI et al., 2020; MORALES et al., 2013). However, in this study, we observed contrary findings (GISPERT et al., 2007; KIM et al., 2020) concerning the higher backfat thickness deposition compared with the Duroc group.

Table 1 – Effect of Duroc, Hybrid and Pietrain finisher breeds on on carcass characteristics..

	Lineages						
	Duroc		Hybrid		Pietrain		*p*-value
	Mean[1]	SE[1]	Mean	SE	Mean	SE	
Initial weight (kg)	20.581	0.950	20.315	0.950	19.90	0.950	0.878
Final weight (kg)	133.461	3.043	134.597	3.043	128.134	3.043	0.297
Hot carcass weight (kg)	97.990^{AB}	0.877	98.446^{A}	0.899	95.243^{B}	0.923	0.030*
Cold carcass weight (kg)	94.824^{A}	0.843	94.915^{A}	0.876	91.802^{B}	0.876	0.018*
Hot carcass yield (%)	73.490 ^{A}	0.313	72.496 ^{B}	0.326	71.897 ^{B}	1.790	0.002*
Loin depth (mm)	82.389^{A}	0.982	82.872^{A}	1.008	79.000^{B}	0.995	0.014*
Backfat thickness (mm)	12.239^{B}	0.634	13.241^{B}	0.651	17.600^{A}	0.660	<0.001***

[1] The values were expressed as means and standard error (SE).
^{A,B} Within the same line, different letters indicate significant differences among the mean values at *** $p \leq 0.001$; * $p \leq 0.05$.

IV. CONCLUSION

The crossbreeding strategy using different terminal sire lines in this study showed an important findings in relation to carcass yield. Our results suggest that the Hybrid pigs may be more advantageous for the production yields in pork industry. Further studies are necessary to better understanding the pork quality.

ACKNOWLEDGEMENTS

This study was funded by the São Paulo Research Foundation (FAPESP, process number 2023/02067-7). We acknowledge the support of the Brazilian National Council for Scientific and Technological Development (CNPq) for the purchase of reagents. We express our gratitude to DanBred Brazil for providing the animals, housing, food, and personnel, which assisted in conducting this research. Additionally, we appreciate the collaboration and provision of space and personnel by the Suinco slaughterhouse.

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Exploring meat quality variations from Duroc, Hybrid, and Pietrain finishing pig lines

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I. INTRODUCTION

Pork is an important source of animal protein and is among the most consumed meats in the world (MOTE; ROTHSCHILD, 2019). Meat quality is influenced by several factors, the genotype being one of the most important. A viable approach within genetic programs to enhance desirable traits in offspring is the introduction of genetic diversity through crossbreeding systems (KIM et al., 2014). Genetics plays an important role in determining the characteristics of muscle tissue, thus influencing the organoleptic properties of meat, such as flavor and texture (LEBRET; ČANDEK-POTOKAR, 2022). Color is a crucial parameter for quality, influencing consumer perception and their purchasing decision. In addition, the attractive appearance of meat is associated with freshness, flavor, and quality (GAGAOUA et al., 2023). Another factor that indirectly influences consumer purchasing decisions is pH, due to its relation to water retention capacity, color, tenderness, and shelf-life of the meat. Based on this, the main objective of this study was to compare meat quality data, especially pH and color, from pigs of different genetic lines.

II. MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use of the Luiz de Queiroz College of Agriculture (University of São Paulo, Piracicaba, Brazil), under CEUA nº. 7416051222. A total of 600 pigs were used for this experiment, representing three sire lineages: Duroc, Pietrain, and hybrid (50% Duroc and 50% Pietrain), of which, in total, 117 animals were randomly selected, with the maternal lineage being the DanBred Hybrid (DB90) from DanBred Brasil, maintained the same for all sires. All pigs were raised in the same management and nutritional system in the same fattening pig farm. Slaughter occurred at 169 days and the left half carcasses were used for to evaluate the quality characteristics of the meat in the *Longissimus lumborum* (LL) muscle. The color (L*, a* and b*) of the LL muscle was assessed using the Konica Minolta colorimeter with calibration parameters provided by the manufacturer (D65, Y= 93,7, X= 0,3160, y= 0,3323), and the final pH was measured using a digital pH meter, after refrigeration. To assess the effects of different crossbreeding systems on pork quality traits, we conducted an analysis of variance (ANOVA) with terminal sire line and slaughter age as a fixed effect and covariate, respectively, followed by the Tukey test. All statistical analyses were performed using the R program, and significance was assessed at p-value ≤ 0.05.

III. RESULTS AND DISCUSSION

The color (L*, a* and b*) and final pH are shown in Table 1. In this study, there was no difference (p > 0.05) on pH and color among the terminal sire line groups. However, there was a trend towards of difference for the b* color parameter (p < 0.10), as shown in Table 1. The Duroc pig showed higher yellowness (b*) and lightness (L*) values than Pietrain and Hybrid animals. The redness (a*) was lower in Duroc pigs and more intense in Pietrain animals. Regarding the final pH, Pietrain and Duroc pigs had the highest and lowest pH values, respectively. Additionally, the pH of Pietrain pigs in this study was 5.68, which was similar to the pH of 5.67 in the Hybrid pig population. The results obtained in this

study indicate that the crossbreeding of finishing pig lines did not have a significant effect on the final pH and meat color parameters. However, there was a tendency ($p < 0.01$) for Duroc pork to exhibit higher yellowness than Pietrain and Hybrid. This slight color difference between the meats may influence the consumer's purchasing decision. These findings corroborate with previous research that highlighted the benefits of crossbreeding pigs in improving meat quality. (KIM et al., 2020). However, in this study, we identified results that are opposite to those found by Edwards, Bates and Osburn (2003), who reported a significant difference between pH values and the color parameter a*.

Table 1 – Effect of Duroc, Hybrid and Pietrain finisher breeds on color and final pH post-mortem.

	Lineages						
	Duroc		**Hybrid**		**Pietrain**		***p*-value**
	Mean[1]	**SE[1]**	**Mean**	**SE**	**Mean**	**SE**	
L*	51.354	0.427	51.239	0.443	51.090	0.443	0.911
a*	4.939	0.199	4.982	0.206	5.105	0.206	0.837
b*	6.075	0.196	5.486	0.203	5.661	0.203	< 0.1
Final pH	5.631	0.023	5.671	0.0242	5.680	0.0239	0.302

[1] The values were expressed as means and standard error (SE).

IV. CONCLUSION

The use of different finishing pig lines for crossbreeding in this study did not show significant differences regarding the color parameters and final pH of meat quality.

ACKNOWLEDGEMENTS

This study was funded by the São Paulo Research Foundation (FAPESP, process number 2023/02067-7). We acknowledge the support of the Brazilian National Council for Scientific and Technological Development (CNPq) for the purchase of reagents. We express our gratitude to DanBred Brazil for providing the animals, housing, food, and personnel, which assisted in conducting this research. Additionally, we appreciate the collaboration and provision of space and personnel by the Suinco abattoir.

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Camelina sativa as an emerging sustainable feedstuff for broiler quails (*Coturnix japonica*): In-depth exploration of the impacts on early post-mortem muscle using shotgun proteomics and bioinformatics

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I. INTRODUCTION

Camelina sativa (CS) is an oilseed crop of the *Brassicaceae* family native to Europe and Southwest Asia. The potentiality of CS relies on its nutritional composition, being rich in proteins (24.5-30%) and lipids (36.5-40.2%) including beneficial omega-3 fatty acids, and on its environmental sustainability. For these reasons, the CS cake is considered as a promising by-product for poultry diets [1]. However, current knowledge on its impact on the muscle and meat traits is unknown. Therefore, this study aimed to explore for the first time the effects of a CS cake incorporated in the diet of broiler quails (*Coturnix japonica*) on their muscle proteome using a shotgun proteomics approach and bioinformatics.

II. MATERIALS AND METHODS

The *in vivo* trial involved 180 of 15-day old broiler quails. The experiment consisted of three dietary treatments (6 replicated cages/treatment and 10 quails/cage): a control diet, consisting in standard growing-fattening diet (0%), and two diets formulated to include 5% and 10% of the CS cake from the ALAN line, genetically improved for a reduced content of glucosinolates [2]. Diets were provided *ad libitum* for 20 days in mash form. After slaughter and within 20 min post-mortem, breast (*Pectoralis major*) meat samples from n=6 male quails/treatment (n=1/replicated cage) were sampled and frozen at -70 °C. For proteomics, total proteins from 150 mg of frozen tissue were extracted as previously described [3]. The protein extracts were used to prepare protein bands using one-dimensional SDS-PAGE for shotgun proteomics using LC-MS/MS [4]. The proteome database (filtering criteria of 2 unique peptides, 10% coverage score and an FDR of 1%) was analyzed using several approaches. For statistical analyses: 1) Partial Least Square-discriminant analysis (PLS-DA), 2) heatmap hierarchical analyses and 3) pairwise comparisons using volcano plot (1.2-fold change and *p*-value 0.05) to identify the differentially abundant proteins (DAPs). For bioinformatics: pathway enrichment analysis (Gene Ontology - GO) using Metascape® as described by Gagaoua *et al.* [5].

III. RESULTS AND DISCUSSION

The comparison of the muscle proteome of quails fed with different inclusion levels (0%, 5%, 10%) of CS cake are given in Figure 1. The PLS-DA discriminated the three treatment groups (Figure 1A). This indicates that the dietary treatment had a remarkable effect on the quail muscle proteome, with the inclusion level being a key factor in explaining the observed differences. The clear discrimination of the treatments was further evidenced at the individual level as depicted in the statistical heatmap (Figure 1B). The overlap analysis in terms of number of proteins that were changed across the groups revealed higher number in the 10% inclusion level than 5%, both higher compared to 0% (Figure 1C). A significant number of proteins were commonly changing among the treatments. This allowed to explore and compare the molecular pathways to which the proteins belong (Figure 1D) for each comparison, focusing on the top 20 GO enriched terms. Briefly, ten GO terms were common to the three comparisons, but more importantly enriched in the control-ALAN 10% comparison. This suggests a link with the CS inclusion in the feed. Interestingly, CS showed a remarkable up-regulatory effect of pathways encoding for endomembrane system organization, lipid biosynthetic process, mRNA metabolic process, Golgi vesicle transport, ribonucleoprotein complex biogenesis, peptidyl-amino acid modification, amide biosynthetic process, response to wounding and protein catabolic process. The results depicted dynamic changes in the muscle proteins, from which certain pathways such as lipid biosynthetic are in line with the chemical properties of CS cake. The changes in the muscle proteome of quail would have consequences on the nutritional and meat quality properties, which need to be investigated though the correlation of the DAPs with intrinsic meat quality traits.

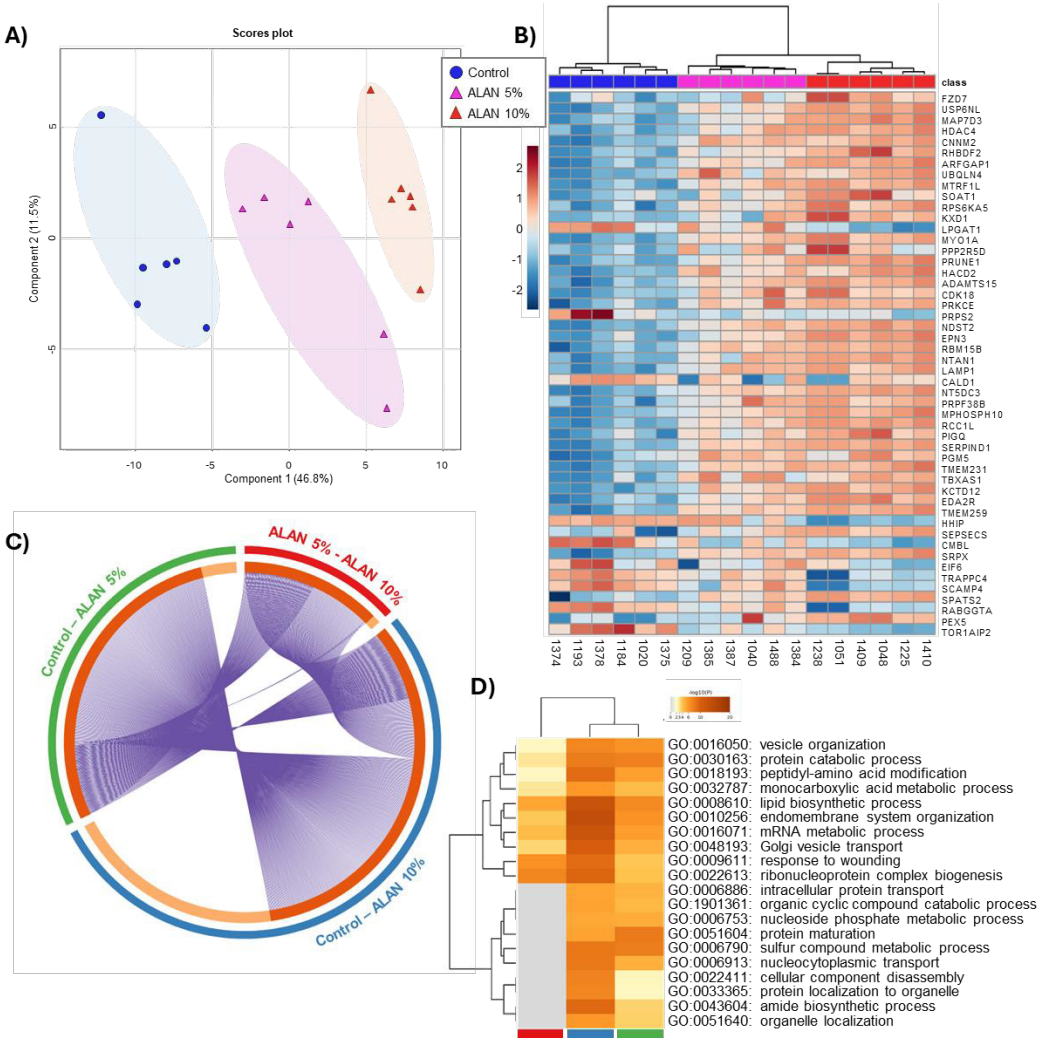

Figure 1. Comparison of quail muscle proteome between the control and after 5% and 10% inclusion of CS cake. **A)** PLS-DA score plot; **B)** Heatmap visualization and dendrogram; **C)** Circos plot depicting the degree of overlap in the proteins across the three comparisons; **D)** Hierarchical Heatmap clustering comparing the degree of enrichment in the molecular pathways using the top 20 significantly enriched GO terms.

IV. CONCLUSION

The findings evidenced that dietary inclusion of CS cake into quail's diet induced dynamic changes in the muscle proteome, which seemed to be directly depended on the inclusion level of the feedstuff.

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CANDIDATE GENES CAST AND CAPN AS POTENTIAL COLOUR BIOMARKERS IN FRESH MEAT

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I. INTRODUCTION

Recent advancements in molecular genetics in animal production have paved the way for the identification of markers associated with specific genes, which significantly influence meat quality [1]. Gene expression analysis is a technique that allows identifying whether a target gene is expressed in a given cell or organism, aiming to understand its relationship with the phenotype of interest studied. The meat colour has always been a topic of extensive research and discussion, given its status as the first characteristic evaluated by the final consumer. However, despite the widespread study of the calpastatin (CAST) and calpain (CAPN) genes and their association with tenderness [2], research on these genes in the context of fresh beef is a relatively new and developing area in the literature. Therefore, the objective of this study was to establish a correlation between the relative gene expression of CAST and CAPN and the colour parameters L*, a*, b*, C*, h*, R630/580, Oxymyoglobin (OMb), Deoxymyoglobin (DMb) and Metmyoglobin (MMb) in fresh meat from Bos indicus animals.

II. MATERIALS AND METHODS

Twenty-four non-castrated male Nelore cattle (*Bos indicus*) were used. The *Longissimus lumborum* (LL) muscles were portioned into 2.5 cm thick steaks and subjected to blooming (oxygenation) for 30 minutes in a refrigeration chamber at 2 ºC (± 2) at three days post-mortem and destined for instrumental colour analysis. LL samples were collected thirty minutes post-mortem to perform gene expression analysis of the CAST and CAPN genes. Real-time quantitative PCR (rt - qPCR) was performed, and relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method [3]. Pearson test (5%) was used to evaluate the relationship between the CAST and CAPN genes with the colour parameters.

III. RESULTS AND DISCUSSION

The results (Figure 1) indicate that the expression of the CAST and CAPN genes can be used to estimate parameters related to meat colour significantly. Both genes showed positive correlations with a*, b*, C*, h* and R630/580. Also was observed a negative correlation of those genes with MMb (%).

The biological mechanisms between the calpain and calpastatin proteolytic system and the colour parameters presented (Figure 1) may be related to the concentration of Ca^{2+} ions and post-mortem muscle contraction. During the maturation process there is an increase in the concentration of calcium ions and the activity of calpain, releasing calcium from intracellular reserves as part of the rigor-mortis process and activating the calpain enzyme through the maturation process [4]. In this way, increased calpain activity could degrade muscle proteins such as myoglobin, directly related to meat colour. We hypothesise that this degradation may affect the stability of myoglobin and its ability to

capture oxygen, influencing the meat colour parameters observed in the present study (Figure 1).

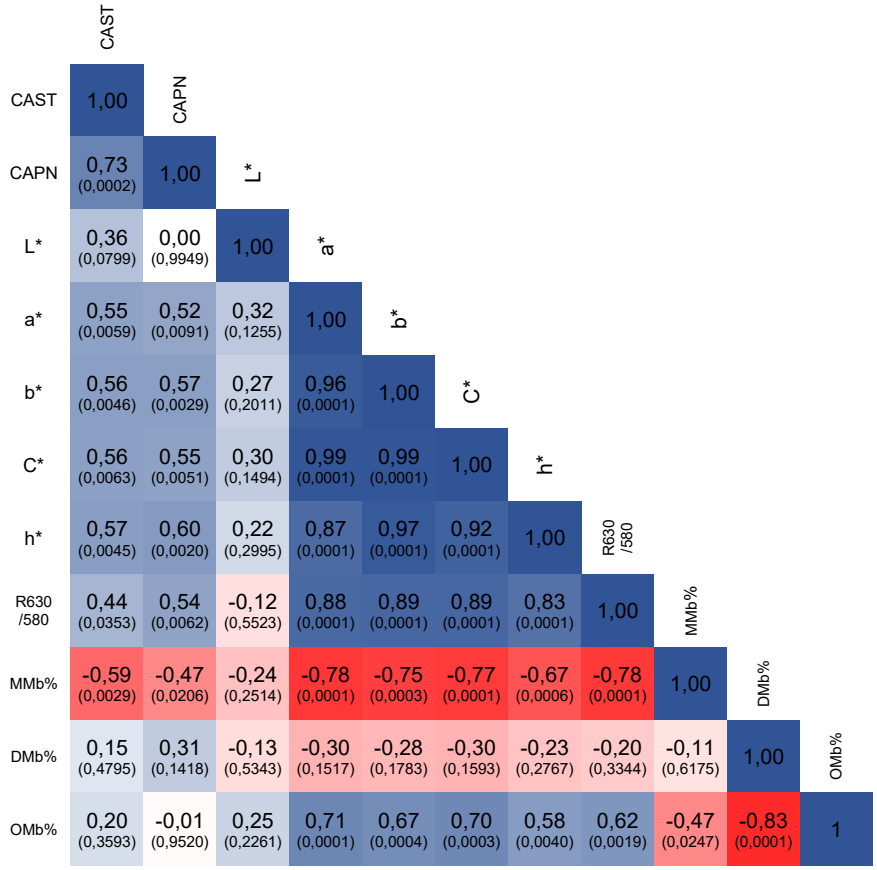

Figure 1. Pearson correlation matrix between CAST and CAPN genes expression, L*, a*, b*, C*, h*, R630/580, MMb%, DMb%, OMb%. The upper value of each cell: Pearson correlation coefficient; the lower value: inferential test

IV. CONCLUSION

The expression of CAST and CAPN genes in *Longissimus lumborum* muscle showed a significant correlation with colour parameters, which are potential candidates genes to indicate important characteristics of fresh meat colour.

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TRANSCRIPTOME PROFILE OF ANGUS-NELLORE CATTLE OF DIFFERENT SEX CLASSES ASSOCIATED WITH CARCASS TRAITS.

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I. INTRODUCTION

Over the last decade, there has been a growing shift in dietary intake patterns, creating a high demand for high-quality, nutritionally valuable meat products [1]. Consequently, studies utilizing advanced molecular techniques have been developed to characterize different meat profiles for a better understanding of the biological events involved in the meat desired by consumers. The use of castration to enhance carcass standardization and meat quality is widely employed. However, its effects on crossbred cattle still present discrepancies regarding their biochemical and molecular mechanisms, particularly in animals subjected to super-early production systems (< 24 months), finished in feedlots for 230 days. Therefore, the aim of this study is to characterize the meat of non-castrated and immune-castrated male cattle through the transcriptome profile of muscle tissue associated with carcass traits.

II. MATERIAL AND METHODS

This study involved twelve F1 Angus-Nellore crossbred animals, averaging 8 months old. Employing a fully randomized design, the animals were divided into two groups: six non-castrated males and six males subjected to GnRH-immunecastration, receiving three doses of the anti-GnRH vaccine at 250, 280, and 370 days of age. The animals were early weaned at 120 days old and housed in collective pens, where they were fed a growing diet until the start of the experiment. During the experimental period, the animals were housed in two pens according to treatment, with an average body weight of 292 kg ± 27 for non-castrated males and 289 kg ± 26 for immune-castrated males. Throughout the experiment, the animals received a growing diet for 110 days followed by a finishing diet for 120 days, totaling a 230-day experimental period. At the end of the experiment, the animals were slaughtered in a commercial slaughterhouse, and samples of the *Longissimus thoracis* muscle fresh were collected after the evisceration and stored in liquid N_2 for RNAseq analysis. After 48 hours of chilling, the carcasses were sectioned at the 12th rib for evaluation of loin muscle area (LMA) and backfat thickness (BFT). Muscle samples from three animals per treatment underwent RNA extraction using a commercial kit (RNeasy Mini Kit, Qiagen, Hilden, Germany). Libraries were prepared using the TruSeq Stranded mRNA kit (Illumina, USA) and sequenced on the NextSeq2000 platform (Illumina, San Diego, USA) with 2x100 bp reads. Only reads above 70 bp and a Phred score of 33 were used for mapping to the *Bos taurus* genome - ARS-UCD.1.2.1. Genes found exclusively in one of the treatments were identified using the DESeq2 package of the R statistical software. Additionally, transcription factor genes were identified through co-expression analysis using the CeTF package in contrast Non-castrated *versus* Immune-castrated. Phenotypic data were subjected to tests for normality of errors (Shapiro-Wilk) and variance homogeneity (Box-Cox) using SAS statistical software version 9.4.

III. RESULTS AND DISCUSSION

Male class significantly influenced carcass traits in the animals (Table 1). Immune castration reduced hot carcass weight by 12% (P = 0.01) and LMA by 14% compared to non-castrated males (P = 0.01). Conversely, immune-castrated males exhibited higher BFT compared to non-castrated males (P = 0.02). Non-castrated cattle showed higher expression of the genes *GH1* (log2FC = 0.47), *LDHC* (log2FC = 1.32), *IDH2* (log2FC = 0.021), *SDHC* (log2FC = 0.096), *UQCRFS1* (log2FC = 0.156), related to carcass traits phenotypes. While immune-castrated cattle had higher expression of the gene *ACOT12* (log2FC = -1.85).

The higher carcass weight and greater protein synthesis in non-castrated animals result from a better utilization of dietary N_2 by this animal category, conferred by testosterone hormone presence [2] associated with higher abundance of growth hormone, encoded by the upregulated *GH1* gene, identified as exclusive in non-castrated animals, demonstrating the intimate relationship between growth hormone and testosterone levels [3]. The greater efficiency and utilization of dietary nutrients are also evidenced by the upregulated gene *LDHC* responsible for lactate conversion to pyruvate, *IDH2* and *SDHC* belonging to the citric acid cycle, and the *UQCRFS1* gene belonging to cytochrome c reductase during oxidative phosphorylation. More productive and efficient animals have higher expression of genes involved in oxidative pathways.

Table 1 – Carcass traits of male non-castrated and castrated F1 Angus x Nellore, super early.

Traits	Male Class		SEM	*P*-value
	Non-castrated	Immune-Castrated		
Hot carcass weight - HCW, kg	353.0	310.0	9.40	0.01
Carcass yield - CY, %	59.0	58.4	0.40	0.51
Loin muscle area - LMA, cm^2	88.5	76.5	2.50	0.01
Backfat thickness - BFT, mm	9.4	12.1	0.64	0.02

On the other hand, animals with higher fat deposition, whether in the carcass, marbling, or both, are less efficient, as the energy cost for depositing 1 gram of fat is higher than that of protein due to its higher water content [4]. The greater backfat deposition in immune-castrated animals is supported by the exclusive gene *ACOT12* responsible for converting acetyl-CoA to acetate. Subcutaneous fat deposition has a greater preference for acetate as a substrate [5].

IV. CONCLUSION

The muscular metabolism of cattle from different male classes diverges in terms of substrate deposition and utilization from the diet. Non-castrated males exhibit greater protein synthesis due to their efficiency in utilizing dietary substrates, supported by higher expression of genes such as *GH1, LDHC, IDH2, SDHC*, and *UQCRFS1*. Conversely, immune-castrated males show increased carcass fat deposition via acetate, as evidenced by higher gene expression of *ACOT12*.

ACKNOWLEDGEMENTS

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THE BIFUNCTIONAL EXPLORATION OF PYRUVATE KINASE IN POSTMORTEM MEAT

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I. INTRODUCTION

Pyruvate kinase (PK) acts as one of the glycolytic rate-limiting enzymes and involves in meat quality regulation. Recent studies have reported that some metabolic enzymes can also be regarded as protein kinase in live tissues [1]. A variety of proteins in mitochondria, cytoplasm and nucleus can be phosphorylated by PK [2]. The role of PK as a glycolytic enzyme or a protein kinase appears to participate in the development of meat quality. The objectives of the present study was to evaluate the bifunctional properties of PK in postmortem meat.

II. MATERIALS AND METHODS

This study includes two parts. First, PK inhibitor (shikonin) was added to lamb meat to evaluate the glycolytic activity of PK through measuring the lactate content and physicochemical traits of meat quality. Second, An vitro system was performed to investigate the kinase activity of PK. PK was mixed with myofibrillar protein in buffer (1 mM PEP, 100 mM KCl, 50 mM MgCl2, 1 mM DTT, 1 mM NaVO4, 5% glycerin, 30 mM HEPES, pH 7.5) and incubated at 4, 25 and 37℃. Protein phosphorylation induced by PK was evaluated. The comparisons between different groups were analyzed by one-way analysis of variance (Duncan's multiple range test) and a t-test ($P < 0.05$).

III. RESULTS AND DISCUSSION

The linear fit was performed to better understand the relationship between the lactate content and PK activity. An insignificant pearson'r of -0.20 showed between lactate content and PK activity (Figure 1A), but the myofibrillar fragmentation index was significantly decreased in 1 d and 5d ($P < 0.05$, Figure, 1B). When stored at 4 h, the absorbance of the PKI group at 540 nm and 580 nm were reduced (Figure 1C). Thus, it was speculated that PK might be involved in meat quality regulation through other pathways besides its glycolytic activity.

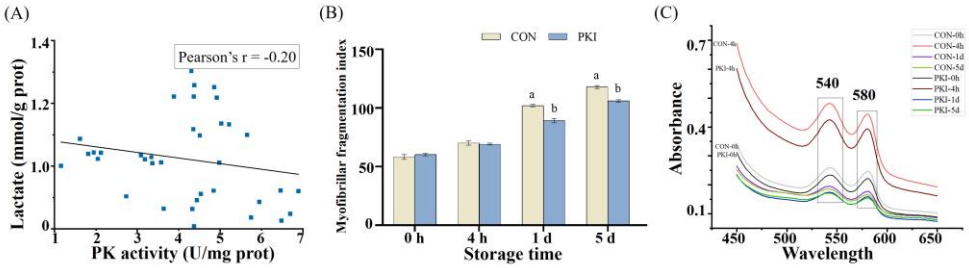

Figure 1. The effect of PK on lactate (A), myofibrillar fragmentation index (B) and myoglobin spectrum (C). a-b shows significant differences ($P < 0.05$). The absorbance at 540 nm and 580 nm represents the oxymyoglobin status.

The kinase assay of PK was conducted to demonstrate whether PK could induce protein phosphorylation. Compared with the control group, the global phosphorylation level of myofibrillar proteins in the PK group was significantly higher at 4℃ for 1 h incubation ($P < 0.05$, Figure 2A)., there was a relative stable change between incubation times ($P > 0.05$, Figure 2B). However, It was

significantly different between the PK group and the control group for 0.5 h and 2 h when incubated at 25℃ ($P < 0.05$, Figure 2B). During the whole incubation at 37℃, the global phosphorylation level of myofibrillar proteins in the PK group was significantly higher compared to that in the control group ($P < 0.05$, Figure 2C). The kinase activity of PK was sensitive to temperature. The findings of a previous study demonstrated that the phosphorylation of myofibrillar proteins inhibited their degradation [3]. Thus, the degradation and phosphorylation of actin and desmin were evaluated (Figure 3). The results showed that both desmin and actin were substrates that could be phosphorylated by PK, especially at the incubation temperature of 37℃. In addition, the actin and desmin phosphorylation induced by PK might take part in their degradation.

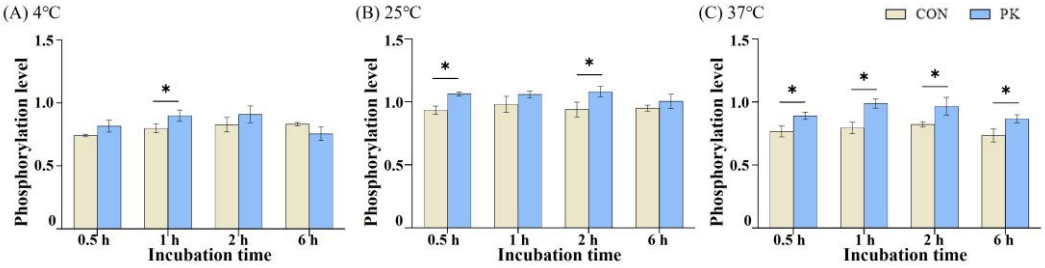

Figure 2. Effect of PK on phosphorylating myofibrillar proteins at 4℃ (A), 25℃ (B) and 37℃ (C) in the control and PK groups. * $P < 0.05$.

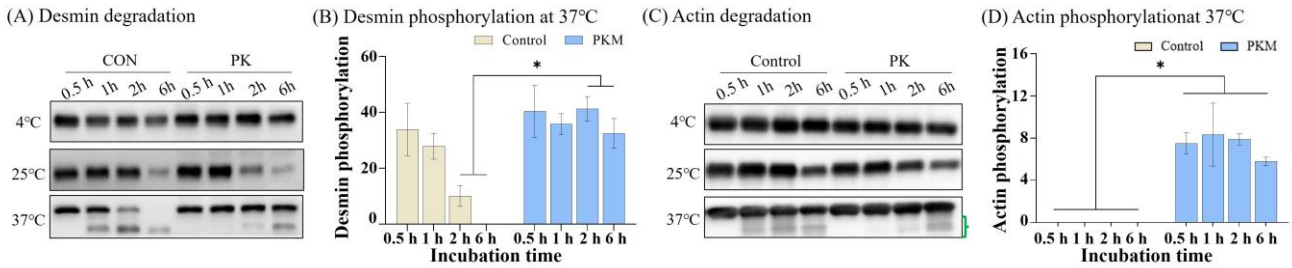

Figure 3. The desmin degradation (A), desmin phosphorylation (B), actin degradation (C) and actin phosphorylation (D) during incubation. The green label in images of (A) and (C) represents the degradation bands. * $P < 0.05$.

IV. CONCLUSION

The role of PK as a glycolytic enzyme and a kinase activity both had effect on meat quality regulation. Especially, the phosphorylation of desmin and actin was increased and the degradation of desmin and actin was decreased after myofibrillar proteins incubated with PK at 37℃. PK induced phosphorylation might be another potential pathway of meat tenderness formation through protein degradation regulation.

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SEX, SIRE AND AGING EFFECTS ON LIPIDS AND PROTEIN OXIDATIVE STABILITY IN COOKED LAMB DURING STORAGE

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I. INTRODUCTION

Dry-aging is a processing strategy to produce meat with high-quality and unique flavors. Dry-aging can be achieved by traditional methods without any packaging or in-bag dry-aging (BD). BD is a relatively new technique to produce dry-aged meat with more consistent quality and increased yields [1]. However, factors affecting the quality and stability of dry-aged meat are complex. The interplay of dehydration, oxidation, and microbial activity has been suggested to affect the development of characteristic dry-aged meat flavor [1], while other factors such as genetics, sire, and sex remain less explored. Certain sires were reported to produce lamb meat with more stable color during storage (color-stable) [2] and such advantages may further influence other quality traits and stability during processing and storage [3]. This study aimed to determine the impacts of sex, sire, and aging type on the oxidative stability of lipids and protein in raw and cooked lamb meat during chilled storage.

II. MATERIALS AND METHODS

Paired hindlegs (n=40) were collected from twenty lambs (~46 weeks) of different sexes (ram/ewe, n=10 each sex) and sire groups (color-stable and color-labile, n=10 each sire) as described by Zhang [3]. The paired legs were randomly assigned wet-aging at -1.5 °C and in-bag dry-aging in a dry-aging bag (Tublin®10) at 2 ± 0.5 °C, 0.5 $m.s^{-1}$ air velocity, and relative humidity of 75 ± 5% for 21 days, resulting 8 treatment combinations (n=5 each) including wet-aging (W) and BD of (1) & (2) male lambs from color-stable sire; (3) & (4) male lambs from color-labile sire; (5) & (6) female lambs from color-stable sire; (7) & (8) female from color-labile sire. Forty chops were produced from aged lamb and cooked *sous vide* at 72 °C for 1 h followed by cooling down in an ice slurry for 1 h. Cooked lamb chops were deboned and ground into mince using a food processor. Cooked mince was placed in a form tray and overwrapped with PVC film and stored in a chiller at 4 °C for up to 28 days. Sub-samples were taken during storage for 0, 7, 14, 21, and 28 days. Lipid and protein oxidation of raw (uncooked) and cooked lamb samples were performed following thiobarbituric acid reactive substances assay (TBARS) and 2,4-dinitrophenylhydrazine (DNPH) method, respectively, as described by Zhang [4]. Results were analyzed by analysis of variance in Genstat, specifying aging type, sire groups, sex, and their interactions as treatment effects with carcass IDs and the side within carcass as blocking factors.

III. RESULTS AND DISCUSSION

Low levels of lipid oxidation were found in raw samples regardless of aging type, sex, and sire group. Raw lamb from BD had higher TBARS than the meat from W ($P=0.058$, Table 1) due to the oxygen permeability of the dry-aging bag to allow for oxidative maturation and produce niche dry-aged meat flavor [1,4]. Cooking and chilled storage increased TBARS levels in all the lamb samples. Aging method was the primary factor driving the differences in TBARS content during the storage with higher levels observed in lamb meat from BD compared to W ($P<0.05$), suggesting the lipid is more susceptible to oxidation in cooked dry-aged lamb during chilled storage. Lamb meat from color-stable sire had lower TBARS values ($P=0.015$) than the labile sire after cooking, while such differences diminished during storage. Sex had no impact on the lipid oxidation in both raw and cooked lamb meat.

Table 1 Lipid and protein oxidation levels of raw and cooked lamb chops during storage for 28 days.

	In-bag dry-aging				Wet-aging				Pr > F		
	Male		Female		Male		Female		Sex	Sire	Aging
	Stable	Labile	Stable	Labile	Stable	Labile	Stable	Labile			
	Lipid oxidation (mg MDA/kg meat)										
Raw	0.42	0.29	0.36	0.40	0.34	0.24	0.28	0.27	0.931	0.331	0.058
Cooked and storage											
0 d	2.04	2.31	1.78	2.43	1.87	2.11	1.88	2.12	0.814	0.015	0.010
7 d	6.45	7.28	7.71	7.09	5.01	5.50	5.31	5.33	0.065	0.245	<.001
14 d	7.84	8.24	8.50	7.96	5.40	6.41	5.55	5.68	0.876	0.441	<.001
21 d	10.00	10.64	11.22	10.64	7.56	7.91	7.78	7.65	0.179	0.745	<.001
28 d	11.75	11.44	12.40	12.19	8.67	8.59	8.59	8.91	0.119	0.596	<.001
	Protein oxidation (nmol/mg protein)										
Raw	2.98	2.74	2.46	2.36	2.58	2.92	2.02	2.35	0.006	0.638	0.177
Cooked and storage											
0 d	2.68	2.43	2.27	2.53	2.58	2.62	2.42	2.55	0.318	0.729	0.420
7 d	3.28	3.32	2.89	2.54	3.39	3.21	2.48	2.77	0.005	0.792	0.702
14 d	2.99	2.88	2.63	2.79	3.37	3.14	2.62	2.83	0.080	0.969	0.065
21 d	3.99	3.43	3.20	3.39	3.96	3.91	3.28	3.03	0.047	0.695	0.265
28 d	4.31	3.02	2.83	3.33	3.56	3.02	2.97	2.77	0.095	0.262	0.373

MDA (mg malonaldehyde/Kg meat); protein carbonyl (nmol/mg protein).

Similar levels of protein carbonyl were observed in raw and cooked lamb meat regardless of sire groups and aging types. A sire group × aging type interaction was observed in raw lamb meat (P=0.046) with higher levels of carbonyls in dry-aged samples from color-stable sire compared to labile sire, while the opposite trend was observed in wet-aged lamb. Such findings suggested different oxidation pathways may be involved for lamb protein from two sire groups under different aging environments. On the other hand, sex was the main factor driving the changes in both raw and cooked lamb meat. Higher protein carbonyl contents were observed in aged samples from male lambs than the females before cooking (P=0.006) and following cooking and storage for 7 (P=0.005) and 21 days (P=0.047), suggesting more oxidatively stable meat may be produced from female lambs.

IV. CONCLUSION

BD enhanced TBARS levels in both raw and cooked lamb confirming the role of lipid oxidation in developing dry-aged meat flavor. Freshly cooked lamb meat from color-stable sire may have different eating qualities compared to labile sire due to the lower lipid oxidation levels. The superior stability of lamb meat from ewe may be beneficial for producing meat with more stable quality during storage.

ACKNOWLEDGEMENTS

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New Insights into Heme Proteins-Mediated Lipid Oxidation in Meat: Mechanisms and Inhibition

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I. INTRODUCTION

Lipid oxidation leading to quality deterioration is a critical issue and a hot topic of research in meat processing and storage [1]. This study, utilizing a multidisciplinary approach that includes model methodologies, molecular chemistry, structural biology, and computational simulations, provides new insights into the dynamic molecular mechanisms of lipid oxidation in meat and develops strategies for its control.

II. MATERIALS AND METHODS

Hemoglobins (Hbs) were prepared from blood using heparin anticoagulant (120 Units/mL) as described previously [2]. Washed muscle from fresh pig, cod, and turkey was prepared as described previously [3]. Muscle structures were examined, and images were taken in a JEM-1011 electron microscope (JEOL, Japan). The covalent binding between quercetin and turkey Hb was determined by ESI-MS as described previously [4]. Quercetin and its quinone form were docked to structure of turkey Hb tetramer using AutoDock vina program. The crystal structure of Hb was retrieved from the protein data bank. Experiments in this study were repeated at least three times.

III. RESULTS AND DISCUSSION

Uncover the molecular mechanisms of heme protein-mediated lipid oxidation in meat: Research has established heme proteins as the principal pro-oxidants in muscle foods [5]. Yet, the critical step associated with lipid oxidation remains unclear due to the concurrent occurrence of several processes, including heme protein autooxidation, ferryl radical formation, hemin release, heme protein crosslinking, and iron release. In this study, we used various models as well as real meat to identify the key steps of heme proteins-meditated lipid oxidation and found that the oxidation and dynamic dissociation of heme iron porphyrin molecules are central molecular drivers of lipid oxidation in meat (Figure 1).

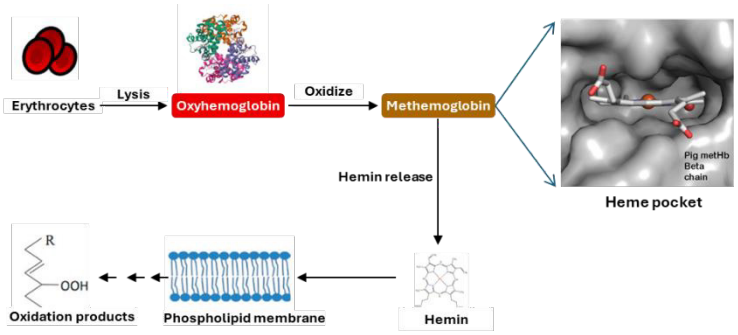

Figure 1. Key processes of heme proteins-induced lipid oxidation in meat products

The role of phospholipids in meat products: From a conventional viewpoint, the amount of phospholipids and the elevated unsaturation of the acyl chains of phospholipids play important roles regarding onset of lipid oxidation in muscle [1]. However, in our study, we observed that adding myoglobin (Mb) increased lipid oxidation in washed cod muscle (WCM) but not in washed pig muscle (WPM) (Figure 2A). Added phospholipids with polyenoic indexes of 282 and 24 activated Mb as an oxidant similarly in WPM (Figure 2B) [6]. The differing microstructure of WCM e.g. more exposed fat cells or membrane of muscle cells compared to the “denseness” or “wrapped” structure of WPM, may have contributed to the better ability of Mb to facilitate lipid oxidation in the WCM.

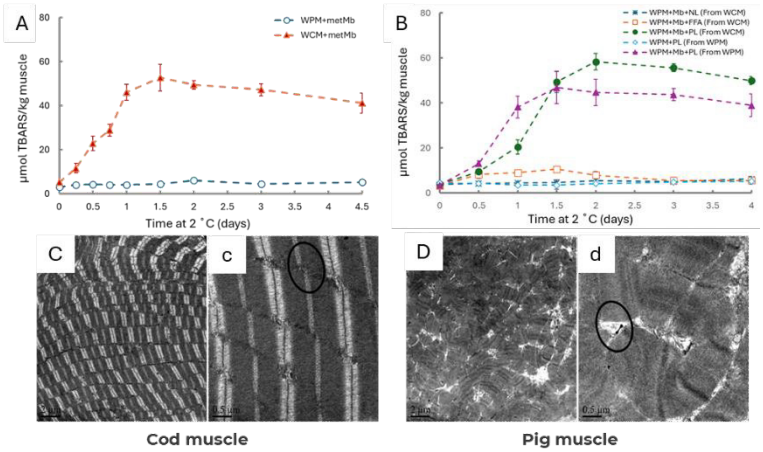

Figure 2. Oxidation performance of phospholipids in cod and pig under the condition of myoglobin (Mb) as a pro-oxidant, along with their muscle structures [6]. Replicates per treatment was n = 3. Means and standard deviations are shown. (A) washed pig muscle and washed cod muscle treated with Mb; (B) washed pig muscle and treated with lipids (NL: Neutral lipids, FFA: Free fatty acids, PL: Phospholipids) and Mb during 2 °C storage.

The antioxidant effect of polyphenols on Hb-mediated lipid oxidation: Numerous studies have demonstrated that free radical scavenging and metal chelation could be the key factors responsible for the antioxidative activities of flavonols. In present study, we found a novel mechanism by which polyphenols with π-conjugated structures, covalently bind to hemoglobin at specific sites near the iron porphyrin, such as the α chain Cys(H15) site (Figure 3). This interaction inhibits the oxidation and dissociation of iron porphyrin molecules, thereby diminishing their pro-oxidative activity [7].

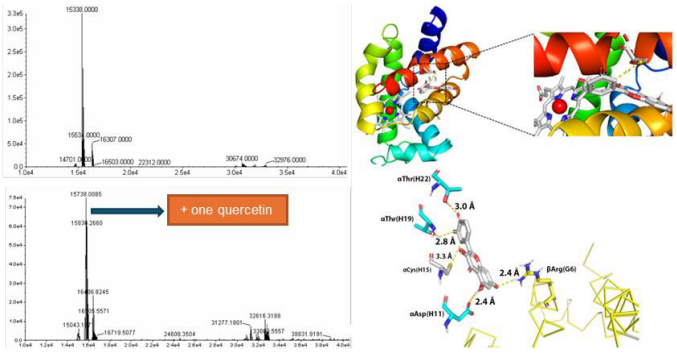

Figure 3. The tetrameric Hb view indicating the binding site of quercetin to Cys(H15) on α-chain of turkey Hb [7].

IV. CONCLUSION

The oxidation and dynamic dissociation of heme iron porphyrin molecules are central molecular drivers of lipid oxidation in meat. Second, the muscle microstructure and the accessibility of heme proteins to phospholipids that are the dominant factors influencing lipid oxidation. Third, the study reveals a novel mechanism where polyphenols covalently bind to hemoglobin near the iron porphyrin, inhibiting oxidation and dissociation of iron porphyrin molecules and reducing their pro-oxidative activity.

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CATTLE TEMPERAMENT AND *LONGISSIMUS* MUSCLE METABOLITES

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I. INTRODUCTION

Modifications in the rate of pH decline is the result of muscle energy *status* in the early *post mortem* period. In the case of *Longissimus* muscle, which is mostly composed of type IIx fibers, greater mitochondrial function and ability to maintain ATP levels during early *post mortem* is associated with delayed pH decline [1]. Animal responsiveness to stress pre-slaughter can affect the muscular ATP balance. Considering that beef from excitable animals has been linked to inferior quality [2], it is important to investigate mechanisms behind the stress physiology in animals with divergent temperaments. In this context, metabolomics is a powerful tool to unravel pathways that are associated with temperament, providing information that can contribute to easily and early access beef with superior quality. Therefore, our objective was to compare *Longissimus* metabolites in the early *post mortem* (1h) from calm and excitable Nellore cattle.

II. MATERIALS AND METHODS

All experimental procedures involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines of the College of Animal Science and Food Engineering at the University of Sao Paulo (6493190121). From a larger group of 72 Nellore males, a sub-group (n = 22) was selected based on temperament tests during the first handling (after animals were transferred from pasture and adapted to feedlot). Chute score and flight speed were determined and averaged to calculate temperament index. The index was used to classify animals either as excitable or calm. Care was taken to select progenies from several bulls in each group. Approximately 1h after slaughter, a small sample was taken from each carcass from the *Longissimus* muscle and between the 12th and 13th ribs, immediately frozen using liquid nitrogen and stored in an ultra-freezer (-80 °C) until processing. Metabolites were extracted using methanol/chloroform/water (2/2/1, v/v/v), as previously described by [3] and analyzed through nuclear magnetic resonance spectrometry (^{1}H-NMR). 1D ^{1}H-NMR spectra were processed, metabolites were identified and quantified using the Chenomx NMR Suite Professional 10.0 software (Chenomx Inc., Edmonton, Canada). Metabolomic data were analyzed using MetaboAnalyst 6.0 (http://www.metaboanalyst.ca/), through Volcano Plot and enrichment analysis.

III. RESULTS AND DISCUSSION

Based on the Volcano Plot, the metabolites that explained most of the data variability were isovalerate, adenine, taurine, glucose, sarcosine, acetate, o-acetylcarnitine and glutathione (Figure 1). The overview of enriched metabolite sets (Table 1) showed that the ‘taurine and hypotaurine metabolism’ was the most significant pathway that helped to differentiate metabolites from the divergent temperaments. This pathway is mostly explained by taurine abundance, which is greater (P = 0.02) in muscle from excitable animals. The second most significant pathway was the ‘glucose-alanine cycle’,

which was mostly explained by glucose and glutamic acid abundance. In this case, d-glucose was greater (P = 0.02) in muscle from excitable animals, while glutamic acid did not differ between groups. The third most significant pathway was the 'transfer of acetyl groups into mitochondria', and the most important metabolites involved were d-glucose, malic acid and adenosine triphosphate (ATP). In this case, the relative abundance of the malic acid and ATP did not differ between groups.

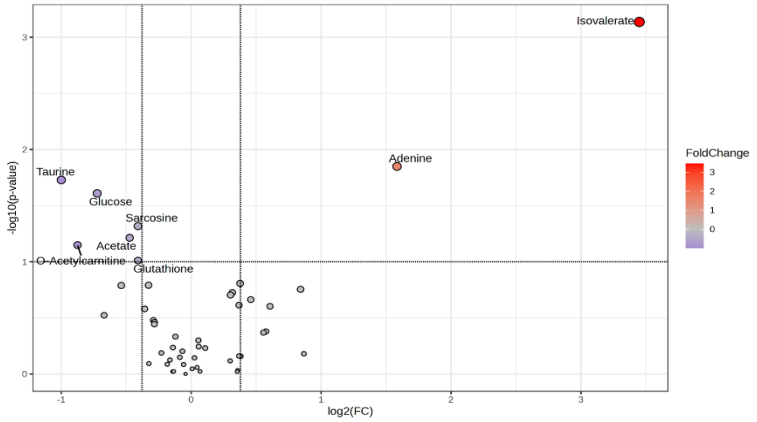

Figure 1. Volcano plot of metabolites from *Longissimus* muscle 1h *post mortem* from Nellore cattle with calm and excitable temperaments

Table 1 – Overview of enriched metabolites sets (top 4) from *Longissimus* muscle 1h *post mortem* from Nellore cattle with calm and excitable temperaments[1].

Pathways	P value	Abundant metabolites
Taurine and hypotaurine metabolism	0.018	Taurine
Glucose-alanine cycle	0.032	D-glucose and glutamic acid
Transfer of acetyl groups into mitochondria	0.046	D-glucose, malic acid, and ATP
Sphingolipid metabolism	0.047	D-glucose and ATP

[1]Temperaments groups were selected based on the temperament index calculated from chute score and flight speed average

IV. CONCLUSION

Nellore cattle classified as excitable have a specific metabolite profile within *Longissimus* muscle at 1h *post mortem* that differs from calm animals. Further research is needed to elucidate the relationship between the profile and early *post mortem* metabolism in bovine with divergent temperaments.

ACKNOWLEDGEMENTS

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Feeding regime alters mitochondrial metabolism in beef muscle

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I. INTRODUCTION

Beef cattle feeding regimes vary greatly depending on country, feed resources and markets. Finishing systems alter whole body fatty acid composition and skeletal muscle energy metabolism impacting meat quality traits, such as color and tenderness [1-4]. Cattle finished in US feedlots are typically fed high carbohydrate diets, which result in maximal average daily gains and bright cherry red lean development. In contrast, beef cattle finished on forage systems are generally fed low energy diets with lower average daily gains and result in darker lean. Differences in diet-induced growth rate also alter skeletal muscle metabolism [2-3]. Regardless, there is a general lack of knowledge regarding the impact of nutrition on mitochondrial substrate utilization. We hypothesized that changes in bovine skeletal muscle mitochondria reflect changes in metabolism as predicated by differences in dietary energy intakes.

II. MATERIALS AND METHODS

Sixteen crossbred Angus steers were randomly assigned to either a forage- or carbohydrate-based maintenance diet for 60 d (n=8). After feeding, cattle were harvested using industry standards. Glycolytic *longissimus lumborum* (LL) and oxidative *masseter* (MS) muscle samples were collected approximately 5 min post-exsanguination. Muscle samples were processed for mitochondrial isolation as described by Scheffler et al. (2014). Mitochondria respiration was measured from freshly isolated mitochondria using Seahorse XFe96 (Agilent). Mitochondria were provided with saturating amounts of the following substrates: palmitoyl-carnitine/malate (40μM/1mM) and acetoacetate/malate (10mM/5mM). Baseline values represent basal respiration of isolated mitochondria with substrates. OXPHOS capacity was determined using ADP (5 mM) stimulated respiration. Proton leak was evaluated using 2 μM oligomycin, while maximal respiration was achieved with the uncoupler FCCP (4 μM). All data were normalized to total loaded mitochondrial protein.

Data were analyzed as a complete randomized design in a 2 x 2 factorial arrangement, considering the diet, muscle, and their interaction as a fixed effect. Harvest date was considered a random effect and animals served as experimental units. Least square means and standard error bars were obtained with SAS Proc Mixed procedure. Significance is denoted as $P<0.05$, $P<0.01$, $P<0.001$, unless otherwise stated.

III. RESULTS AND DISCUSSION

To understand the impact of nutrition on mitochondiral substrate utilization, saturating concentrations of long chain fatty acid (palmitoyl-carnitine) or short chain fatty acid (acetoacetate) were provided to isolated mitochondira to assess their oxygen consumption rate. In the presence of long chain fatty acids, higher maximal respiration were observed in mitochondira isolated from MS of forage fed cattle, followed by those from the MS of carbohydrate-fed cattle, while LL mitochondria had the lowest maximal respiration regardless of feeding regime (Table 1). These data show that feeding regime alters mitochondrial function in the presence of long chain fatty acids and suggest that mitochondria of oxidative muscles are more metabolically malleable compared to those of cattle fed a high carbohydrate diet. Whether this ability is due to the availability of carnitine transporters on the mitochondrial membrane remains to be determined.

Table 1. Mitochondria oxygen consumption rate (pmol/(sec*mg mito)) isolated from the *longissimus lumborum* (LL) and *masseter* (MS) of cattle fed a forage- or carbohydrate-based diet and incubated under saturating concentrations of palmitoyl carnitine/malate.

	Carbohydrate		Forage		p-value		
Injection	LL	MS	LL	MS	TRT	Muscle	T*M
Baseline	20.2±5.5	14.5±5.5	20.6±5.5	9.1±5.5	0.6571	0.1296	0.5991
OXPHOS Capacity	69.3±12.7	223.3±12.7	74.1±12.7	242.3±12.7	0.3601	<.0001	0.582
Proton Leak	8.2±4.9	8.6±4.9	6.7±4.9	8.9±4.9	0.9045	0.7857	0.8536
Maximal Respiration	31.1±13.1	172.8±13.1	39.3±13.1	239.8±13.1	0.0074	<.0001	0.0324

Table 2. Mitochondria oxygen consumption rate (pmol/(sec*mg mito)) of isolated from the *longissimus lumborum* (LL) and *masseter* (MS) of cattle fed a forage- or carbohydrate-based diet and incubated under saturating concentrations of acetoacetate/malate.

	Carbohydrate		Forage		p-value		
Injection	LL	MS	LL	MS	TRT	Muscle	T*M
Baseline	9.2±7.52	BD±7.52	3.8±7.52	BD±7.52	0.1917	0.0016	0.5402
OXPHOS Capacity	26.9±9.7	77.3±9.7	23.9±9.7	136.2±9.7	0.0073	<.0001	0.0034
Proton Leak	9.3±7.2	2.6±7.2	6.9±7.2	BD±7.2	0.3984	0.1586	0.5928
Maximal Respiration	9.7±9.9	80.38±9.9	9.5±9.9	150.8±9.9	0.0014	<.0001	0.0013

When isolated mitochondria were provided saturating concentrations of short chain fatty acids, a treatment by feed effect was noted in OXPHOS capacity and maximal respiration (Table 2). Both assays show that mitochondria from the MS of forage fed cattle have greater respiration than those of carbohydrate fed cattle, with LL mitochondria from either feeding treatment having the lowest respiration. Together these data show that oxidative muscle mitochondria are more responsive to feeding regime than their glycolytic muscle counterparts. Isolated mitochondria from oxidative muscle of may have the ability to utilize more long chain and short chain fatty acids, suggesting that they are more metabolically flexible in terms of substrate utilization.

IV. CONCLUSION

Together these data show that oxidative muscle mitochondria are more responsive to feeding regime than those from glycolytic muscles and that oxidative mitochondria from forage fed animals are able to respire more long chain and short chain fatty acids compared to oxidative mitochondria of carbohydrate fed animals. Future directions will involved studying the mechanisms responsible for this change in mitochondria metabolism.

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EFFECT OF PROGENY ON BEEF QUALITY OF REPRESENTATIVE BULLS WAGYU KUROGE BREED IN TROPICAL CONDITIONS

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I. INTRODUCTION

The inclusion of different breeds, such as the Wagyu Kuroge breed, has been a strategy to improve the quality of the country's beef and meet the needs of more demanding consumers. Wagyu cattle originate from native Japanese breeds and intense marbling tends to be their most notable characteristic [1]. However, different climatic conditions generate physiological and metabolic changes in animals, which can affect beef production in terms of yield, composition and beef quality attributes [2]. In this way, it is suggested that progenies of bulls from the main families of the Wagyu Kuroge breed, raised in tropical conditions, influence the physicochemical characteristics of the beef. Therefore, the aim of this study was to evaluate the influence of the progeny of representative Wagyu Kuroge bulls on the characteristics of tenderness and fat deposition when raised in tropical conditions.

II. MATERIALS AND METHODS

Forty progenies of representative bulls from the main Wagyu Kuroge lines (100% Tajima, Hiroshima, Itozakura, High % Tajima) were used, with 10 progenies from each line. The progenies were castrated, confined (initial mean weight of 450 kg) and maintained on the same diet for 666 days during the finishing phase, and harvested at an average age of 40 months. The criteria for slaughtering the animals were age and final weight. The final mean weight was 732.18 kg, 789.29 kg, 709.75 kg and 784.48 kg for 100% Tajima, Hiroshima, Itozakura and Alto % Tajima, respectively. During boning, after 48 hours, the *Longissimus thoracis* muscle was analyzed for pH, subcutaneous fat thickness and marbling score (Japan Meat Grading Association). Samples of the *Longissimus thoracis* muscle were collected for the analysis of shear force (aged for 2 and 14 days at 0 to 2ºC) and sarcomere length. The procedures for the analysis of shear force were performed according to AMSA (2016), and the sarcomere length was performed according to Battaglia et al. (2016). The lineage effect for all variables was analyzed considering a completely randomized experimental design. Data was submitted to analysis of variance (ANOVA) using the MIXED procedure of the SAS® and the mean results were compared using the Tukey test with a significance level when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

The results are shown in Table 1. For pH values, the lineage did not differ ($P = 0.127$), and the values are within the normal range. Regarding shear force in aged for 2 days samples ($P = 0.299$) and in aged for 14 days samples ($P = 0.314$) there was no difference among treatments, however in aged samples the 100% Tajima, Itozakura and Hiroshima progenies are classified as very tender according to the Ranking of Beef Muscles for Tenderness [5], however, regardless of the lineage, the beef of the progenies stood out for presented a high degree of tenderness.

In relation to the marbling score, there was a difference ($P = 0.008$), where the 100% Tajima and Hiroshima progenies presented higher values when compared to the other lines, corroborating the finding in the literature, which shows that this characteristic is correlated mainly with predisposing genetic factors, either between breeds or between strains [6;7]. As for subcutaneous fat thickness,

there was a difference (P = 0.044), with progenies of the Hiroshima line showed lower values when compared to the others, but all the progenies, regardless of the lineage, revealed values higher than those required for the gourmet market and suitable for the Japanese standard [1;6;8].
There was no difference in sarcomere length (P = 0.093), but the values obtained are in accordance with the literature for beefs considered to be tender, which was confirmed by the values found for shear force, and explained by an adequate decline in pH and appropriate thickness of subcutaneous fat to prevent cold shortening [9].

Table 1 – Influence of the progeny of representative bulls from the main families of the Wagyu Kuroge breed on beef physicochemical characteristics

Variable	Lineage				Pr > F	
	100% Tajima	Itozakura	Alto % Tajima	Hiroshima	SEM	P-value
48h carcass pH	5.53	5.55	5.56	5.33	0.054	0.127
Subcutaneous fat thickness, mm	29.96[a]	29.98[a]	27.36[a]	12.84[b]	4.109	0.044
Marbling score	6.67[a]	4.89[b]	4.92[b]	7.92[a]	0.735	0.008
Shear Force (aged for 2 days), N	46.93	48.60	55.97	41.42	0.306	0.299
Shear Force (aged for 14 days), N	34.88	37.65	43.76	37.16	0.147	0.314
Sarcomere length, µm	2.00	2.17	2.08	2.17	0.180	0.093

[a-b] Averages with different superscripts differ in the rows by the F test

IV. CONCLUSION

Subcutaneous fat thickness and intramuscular fat deposition were influenced by the genetics of the progenies; however, important attributes for beef quality, such as beef tenderness, were not influenced by this factor. Therefore, all the lineage of Wagyu Kuroge progenies raised in tropical conditions have a high-quality final product, giving them an advantage in gourmet markets.

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CATTLE TEMPERAMENT CHANGES NELLORE *TRICEPS BRACHII* METABOLISM

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I. INTRODUCTION

Animals with more excitable temperament or aggressive behavior are more susceptible to diseases and may exhibit inferior performance, reproductive efficiency and poorer meat quality [1,2]. All these physiological changes are derived through alterations in basal metabolism and metabolic rates. It is well established that metabolites, particularly hormones (cortisol), are correlated with excitable temperament in animals. However, variations in metabolic rates and pathways can extend beyond this initial correlation. In this context, metabolomics serves as a potent tool to uncover pathways linked to temperament, providing a clearer understanding of the metabolic processes underlying stress and behavior. This, in turn, can influence meat quality in a more comprehensive manner. Therefore, our objective was to evaluate whether differences in cattle temperament alter the metabolites of the *Triceps brachii* muscle in the early post-mortem period (1h).

II. MATERIALS AND METHODS

The experimental procedures were conducted in accordance with the Institutional Animal Care of the College of Animal Science and Food Engineering at the University of Sao Paulo (6493190121). Twenty-two animals were selected from a larger group of 72 Nellore males, based on temperament tests during the first handling (after animals were transferred from pasture and adapted to feedlot). Chute score and flight speed were determined and averaged to calculate temperament index, which was used to classify animals either as excitable or calm. After finishing, animals were slaughter and approximately 1h after latter, a small sample was taken from each carcass from the *Triceps brachii* muscle, immediately frozen using liquid nitrogen and stored in an ultra-freezer until processing. Metabolites were extracted as previously described by [3] and analyzed through nuclear magnetic resonance spectrometry (^{1}H-NMR). 1D ^{1}H-NMR spectra were processed, metabolites were identified and quantified using the Chenomx software v 10.0 (Chenomx Inc., Edmonton, Canada). Metabolomic data were analyzed using MetaboAnalyst 6.0 (http://www. metaboanalyst.ca/), through Volcano Plot and enrichment analysis.

III. RESULTS AND DISCUSSION

Excitable animals presented a greater glycerol and creatine on muscle early after slaughter (Figure 1). Glycerol is part of the metabolism of lipolysis, indicating an extensive use of energy by those animals, eighter from carbohydrate or lipids. On the other hand, calmer animals presented a greater carnosine and acetate in the muscle (Figure 1). Carnosine plays a versatile role in cell metabolism, including cellular redox reactions and antioxidative, which in turns may influence the establishment of *port mortem* and further, meat quality [4]. The overview of enriched pathways showed most pathways correlated to the aminoacids and energy metabolism differentiating groups (Figure 2).

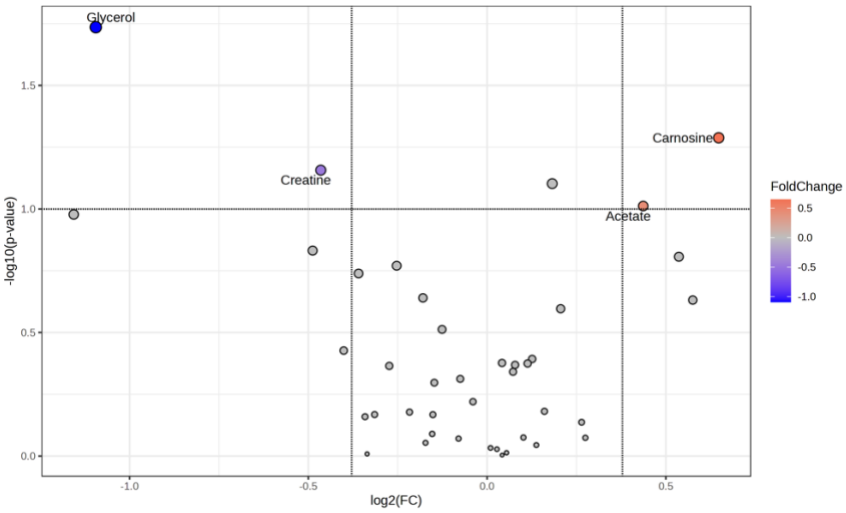

Figure 1. Volcano plot of metabolites from *Triceps brachii* muscle 1h *post mortem* from Nellore cattle according to the temperament scores

Metabolite Set	Total	Hits	Statistic	Expected	P value	Holm P	FDR
Phenylalanine and Tyrosine Metabolism	27	2	13.506	5.2632	0.070477	1.0	0.46962
Aspartate Metabolism	35	5	9.8568	5.2632	0.082719	1.0	0.46962
Inositol Metabolism	30	2	12.143	5.2632	0.096322	1.0	0.46962
Inositol Phosphate Metabolism	24	2	12.143	5.2632	0.096322	1.0	0.46962
Phosphatidylinositol Phosphate Metabolism	17	2	12.143	5.2632	0.096322	1.0	0.46962
Ethanol Degradation	19	1	14.529	5.2632	0.097285	1.0	0.46962

Figure 2. Overview of enriched pathways differentiating groups

IV. CONCLUSION

Nellore cattle classified as excitable presented greater catabolism of lipids and lower antioxidant power compared to calmer animals. Those changes om metabolites early *post mortem* can affluence meat properties.

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METABOLOMIC FINGERPRINTING OF NELLORE CALVES WITH GENETIC VARIATION FOR MEAT TENDERNESS

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I. INTRODUCTION

Nellore (*Bos taurus indicus*) is the most representative breed in the Brazilian cattle herd. However, these animals have less tender meat compared to taurine breeds [1]. Within the breed itself, there is great variation for this trait, which creates an opportunity for research and selection of Nellore animals with more tender meat. This characteristic has been increasingly valued by consumers. Tenderness has been a target of selection in genetic improvement programs, but there is a continuous need to integrate new evaluation and prediction methods to meet consumer market trends. Metabolomics emerges as an alternative to better understand the metabolism of meat tenderness, allowing for the development of new technologies to evaluate and predict tenderness. Therefore, the aim of this study was to assess whether genetic selection of bulls for meat tenderness influences the metabolism of their progeny at birth.

II. MATERIALS AND METHODS

The experimental procedures were conducted in accordance with the Institutional Animal Care of the College of Animal Science and Food Engineering at the University of Sao Paulo (9249180123). One hundred male Nelore calves were divided into two groups based on the genetic variation of the bull (progenitor), selected through expected difference in progeny (EPD) for meat tenderness: Higher EPDs (Tender, n=50) and Lower EPDs (Tough, n=50). At birth, blood samples were collected and subjected to the protocol described by [2], and the resulting filtrate was taken for nuclear magnetic resonance spectroscopy (^{1}H-RMN). The spectra were uploaded to the web tool NMRProcFlow (version 1.4.24, https://nmrprocflow.org/), processed and divided into uniform bucketings of 0.05 ppm width, totaling 112 buckets, which were used for statistical analysis. The data were analyzed in the web tool MetaboAnalyst (Version 6.0, https://www.metaboanalyst.ca/MetaboAnalyst/) using principal component analysis (PCA) and Volcano Plot.

III. RESULTS AND DISCUSSION

There was an overlap between the groups in the PCA analysis, indicating similarity between them. However, in the Volcano plot, it was possible to observe some metabolites responsible for differentiating the groups (Table 1 and Figure 1). Animals in the tender group showed a higher concentration of lactate (P = 0.03), which may indicate greater substrate availability for energy production through gluconeogenic pathways [3]. In contrast, the tough group exhibited higher concentrations of threonine (P = 0.01) and ribose (P = 0.01), suggesting protein and carbohydrate catabolism aimed at energy production [4]. Generally, metabolites have been used to differentiate the degree of tenderness in meat post-slaughter [5,6]. However, in our study, these differences have not yet been correlated with tenderness, but rather with genetic selection for this trait. This could potentially

lead to a deeper and more comprehensive understanding of meat tenderness development from early stages of life.

Table 1 –Metabolites found in the tender and tough treatments.

Metabolites	FC	Log2(FC)	P value	-log10(p)
Threonine	1.5307	0.61417	0.01279	1.8931
Ribose	1.5642	0.64544	0.013142	1.8813
Lactate	0.69936	-0.5159	0.030317	1.5183
Citrate	0.73732	-0.43965	0.079036	1.1022
Tyrosine	0.70248	-0.50948	0.090928	1.0413

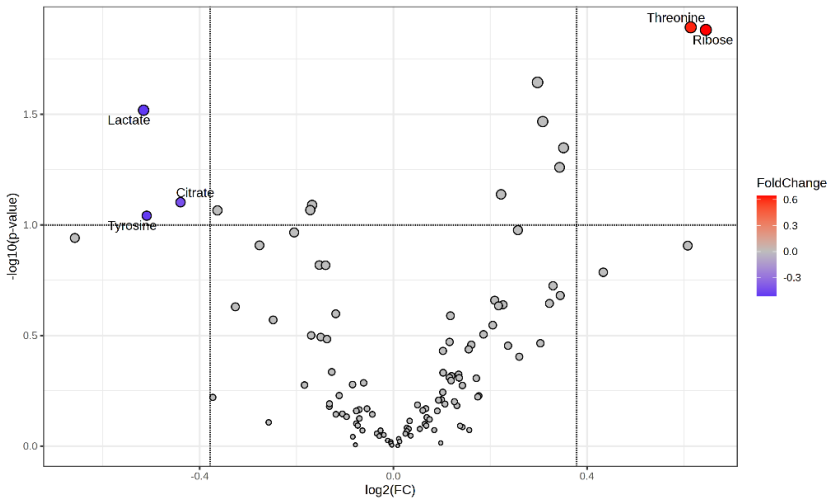

Figure 1. Volcano plot of Nellore calves at birth in the tender and tough groups.

IV. CONCLUSION

Genetic variation for meat tenderness influenced the metabolic fingerprint of the serum of Nellore calves at birth.

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The protein profile in the *longissimus thoracis* muscle from Nellore bulls harvested with different weights reveals proteins that may be related to variations in beef color

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I. INTRODUCTION

The beef color is the meat quality property that most influences consumers' purchase decisions [1]. Diet and production systems can considerably influence final carcass weight and, consequently, affect final pH and meat color. Among the beef color parameters, redness is considered the most important for acceptability criteria [2]. Meat obtained from carcasses with an unfavorable color may suffer economic penalties and, therefore, represent a financial loss for producers and the beef industry. The goal of this study was to evaluate the beef color of cattle harvested at two slaughter weights and similar age by differential proteomics in the *longissimus thoracis* muscle, using the strategy Label-Free Proteomics.

II. MATERIALS AND METHODS

Sixteen Nellore bulls with the same physiological maturity separated into groups according to final carcass weight: Light (127.32±1.92, N=8) and heavy (152.68±1.11kg, N=8) were used. *Longissimus thoracis* samples were collected after slaughter and stored in liquid nitrogen to perform differential proteomics analysis. After a 24-hour chilling period, the pHu was measured (pH meter Testo 205, Lenzkirch, Germany) and 2.54 cm thick steaks were obtained from the *Longissimus* muscle between the 12^{th}-13^{th} ribs for meat color analysis. The meat color measurement was obtained using a Hunter MiniScan EZ colorimeter (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA). The experimental design was a completely randomized with eight repetitions per treatment, and each animal was considered an experimental unit. The SAS software (9.4; SAS Institute Inc., Cary, NC, USA) was used to analyze the data. To evaluate the effects of weight (Light and Heavy) on color parameters, the PROC GLM procedure was applied. The significance was considered when $P \leq 0.05$. The raw data collected by the mass spectrometer was converted into mzXML (extensible mark-up language) files using CompassXport software, version 3.0 (Bruker Daltonics, Germany). In this conversion, the mass/charge ratio (m/z) values were encoded using 64-bit precision. The PEAKS software, version 8.5 (Bioinformatics Solutions Inc., Canada) was used to process the mzXML files, using the PEAKS DB procedure [3] to identify the proteins present in the samples.

III. RESULTS AND DISCUSSION

There was a difference in the a* color component ($P = 0.006$), indicating a redder color for cattle in the heavy group. Similarly, chroma, the color saturation index, was also higher for the heavy group ($P = 0.019$; Table 1). Proteins identified in the meat of Nellore bulls with different carcass weights have been enriched for the following pathways: Metabolic pathways, Oxidative Phosphorylation, Electron Transport Chain, Glycolysis/Gluconeogenesis, Amino Acids Biosynthesis, and Muscle Contraction (FDR ≤ 0.05; Table 1). A total of 22 proteins were identified only in the muscle of the “Light carcass”

group, including Phosphopyruvate hydratase (ENO2) and Stress-70 protein (HSPA9), which are reported as putative protein biomarkers correlated with beef color traits [4].

Table 1 – Meat pH and color parameters of Nellore bulls with different carcass weights and distribution of proteins identified only in meat from the "light carcass" group according to their shared function in different biological processes enrichments in the Gene Ontology network (GO).

	LIGHT			HEAVY			*P-value*
	Mean ± SEM	Min	Max	Mean ± SEM	Min	Max	
CW, kg	125.17 ± 1.83	116.10	133.10	150.41 ± 1.06	145.5	155.5	<0.001
pHu	5.66 ± 0.05	5.61	5.77	5.64 ± 0.05	5.54	5.70	0.482
L*	35.47 ± 1.05	29.47	40.97	36.18 ± 1.00	32.26	40.77	0.632
a*	13.93 ± 0.46	11.52	15.82	15.85 ± 0.39	14.00	17.69	0.006
b*	12.47 ± 0.44	9.14	13.48	13.52 ± 0.58	10.39	15.85	0.167
Chroma	18.74 ± 0.52	16.04	20.42	20.87 ± 0.63	17.45	23.23	0.019

Biological Process[1]	**Gene**	**FDR[2]**
Mitochondrial electron transport, NADH to ubiquinone	NDUFS1; NDUFA8; NDUFA7	0.009
Pyruvate metabolic process	DLAT; ENO2; GAPDHS	0.018
Oxidative Phosphorylation	NDUFS1; ATP5PD; NDUFA8; NDUFA7	0.009
Aerobic respiration	SUCLG1; NDUFS1; ATP5PD; NDUFA8; NDUFA7	0.001
Generation of precursor metabolites and energy	GAPDHS; ENO2; SUCLG1; NDUFS1; ATP5PD; NDUFA8; NDUFA7	0.000
Cellular metabolic process	TUFM; HSPA9; ALDH7A1; AKR1B1; GAPDHS; ENO2; SUCLG1; NDUFS1; ATP5PD; NDUFA8; NDUFA7; DLAT	0.001

[1]Biological Process (Gene Ontology). Software String 12.0. [2]False Discovery Rate – *P-value* corrected for multiple tests within each category using the procedure by Benjamini & Hochberg (1995).
CW = carcass weight; SEM = standard error of the mean; Min = minimum; Max = maximum.

IV. CONCLUSION

This study indicates that heavier cattle of the same age compared to lighter ones tend to yield beef with greater a* and chroma attributes while having no difference in final pH. Standardizing harvesting weight can mitigate color variations in meat from animals with normal pH levels. Furthermore, proteins associated with the oxidative pathway may influence the coloration of beef from cattle of different weights.

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MEAT AND CARCASS QUALITY OF ROMOSINUANO CREOLE CATTLE CROSSED BY BRAHMAN COMING FROM A PRODUCTIVE SYSTEM OF SINÚ VALLEY, CARIBBEAN REGION, COLOMBIA.

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I. INTRODUCTION

One of the priority goals of the Colombian government's policy is to promote beef cattle ranching under sustainable agrosilvopastoral systems. Since 2013, the Colombian Corporation of Agricultural Research (Agrosavia), Turipaná research center (RC), has been working in a productive model for the Caribbean region [1]. This model is based on free agrochemical forages, low tropic conditions, hot weather, high humidity, and dry season. The model also promotes the use of creole animals and their crosses, that are well adapted to this environment. Turipaná RC has the nation´s germplasm bank of the *Bos taurus* Romosinuano breed. This breed and their crosses have great advantages when compared to other Mercosur cattle: it produces meat with Taurus-type quality in difficult environmental conditions, it can reduce the fattening cycle to around 2 years, which favors meat attributes associated with tenderness, and their meat has lower fat content compared to other breeds, which makes it healthier, among other aspects. Most of the meat quality studies on this creole breed has been made outside of Colombia, in feedlots systems in North America [2]. The aim of this study was to characterize the carcass and meat quality of crossbreed Romosinuano by commercial Brahman fattened at Sinú Valley in a productive model for Caribbean region of Colombia.

II. MATERIALS AND METHODS

Ten grass-fed crossbreed males of 19m of age (aprox), 419.1± 16.4kg, were fattened at Turipaná RC until slaughter (525±28,9kg). Animals were in a paddock (rotational system) with different bushes and trees, and grazing *Megathyrsus maximus* cv Sabanera by 149 days at a stocking rate of 4 animals/he. During dry season, the animals were supplemented under grazing conditions with local products: a mixture of corn bran (*Zea Mays*) (65%), Cotton cake (*Gosypium sp*) (25%), and Molasses (*Saccharum officinarum*) (10%), and the amount to supplement consisted of 0.4% of live weight, by 18 weeks. The animals received mineralized salt and water ad libitum during all the fattening process. Animals were slaughter at INVIMA authorized commercial abattoir. Carcass weight (hot and cold, HCW and CCW, respectively), yield (%), backfat thickness (mm) and pH24h were evaluated on the carcass. Four commercial cuts (Tenderloin, Striploin, Rump Cup, Rost Biff) with 10d of ageing were evaluated in terms of nutritional and instrumental values: humidity, total ash, crude fat, crude protein; instrumental color, Warner-Bratzler shear force of cooked meat and cooking losses. To determine histologically the length of the sarcomere [3], samples of 1.5 x 1x 0.5cm (length x width x thickness) were removed from the cuts without ageing (24 h after slaughter). Descriptive statistic was carried out in which the average, minimum and maximum value are shown. Meat cuts was compared between them. The effect of the type of cut (muscle) was carried out by ANOVA using the method of least squares under a completely randomized design. For significant effects, Tukey's multiple comparison test was used. In the case of the variables evaluated in the sensory tests, the non-parametric Friedman rank test was used and the comparison between cuts was made based on the median and the number of samples with scores above or below said reference. Data were analyzed using the SAS Enterprise 8.3 program at a significance level of 5%.

III. RESULTS AND DISCUSSION

Related to carcass trait the HCW, CCW, yield (hot and cold%), backfat thickness (mm), pH24h and temperature were 283.17±22.4kg (259.2- 326.8kg), 281.24±22.4kg (258- 325.1kg), 53.89±1.7% (51-57.4%), 53.52±1.7% (50.8- 57.1), 4.8±1.6mm (1- 6mm), 5.69±0.2 (5.1- 5.9), and 4.72±0.8°C (3.9-5.8°C) respectively. Compared with national indicators for bovine males (DANE, 2023), the CCW of animals coming from this model were 26.6kg higher; however, the cold yield was 0.52 percent point lower. It is important to highlight that these creole crosses were slaughter younger (aprox. 24month) than the average age to slaughter in Colombia (40 month), which helps to reduce the meat production cycle. Most of the beef cattle in Colombia have an *indicus* origin, which have a larger biotype and later maturation than this creole *bos taurus.*

Table 1 – Histological, nutritional and instrumental characterization of 4 cuts of meat from Romosinuano cattle beef crossed with commercial Brahman.

	Tenderloin (*Psoas maior*)	Striploin (*Longissimus thoracis*)	Rump cup (*Gluteus biceps*)	Rost Biff (*Gluteus medius*)
Sarcomere length*	3.32a ±0.09	1.90b ±0.09	1.80b ±0.09	1.71b±0.09
Crude protein (g/100g)	21.29±0.26	22.05±0.32	22.04±0.44	22.51±0.55
Crude fat (g/100g)	2.02±0.31	1.66±0.25	2.04±0.1	1.54±0.16
Moisture (%)	75.43a±0.37	75.21b±0.34	74.98b±0.35	74.1b±0.39
Total ash	1.11ab±0.03	1.03b±0.02	1.18a±0.05	1.17a±0.04
Cooking losses (%)	.32±0.01	.31±0.01	.32±0.0	.28±0.01
Shear force (KgF)	3.8±0.1	3.96±0.21	3.83±0.33	4.24±0.13
pH	5.75±0.05	5.66±0.04	5.61±0.03	5.71±0.12

*Without ageing. Different letter means statistical differences between cuts (p≤0.05)

Meat quality traits for four Romosinuano beef cuts are showed in table 1. There were no significant differences in most of meat quality parameters, and in general those values are within the normal range for beef. It is important to highlight the significant differences (p≤0.05) in sarcomere length and moisture, where tenderloin obtained the higher values. Although, there were no significant differences en shear force between cuts, the values found are quite interesting. On the other hand, these cuts show a lower level of fat, something that may be characteristic of Romosinuano meat.

IV. CONCLUSION

The implementation of a productive model plus the use of the Creole breed or its crosses, can contribute significantly to meat production in the Caribbean region of Colombia, from the point of view of reducing the productive cycle, as well as in its contribution to the quality of meat associated with taurus breeds.

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Omics strategies to differentiate *Longissimus lumborum* beef within three ultimate pH groups

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I. INTRODUCTION

The influence of ultimate pH (pHu) ranges on quality parameters of aged *Longissimus lumborum* steaks from Nellore bulls has been studied (Barón et al., 2021; Lomiwes et al., 2013; Ramanathan et al., 2020). However, there is a gap of omics studies applied for intermediate pHu (5.8-6.2) muscles aged for long periods. Our objective was to assess the changes on lipidome and metabolome profiling of *Longissimus lumborum* beef of Nellore bull muscle with different pHu (normal [< 5.8], intermediate [5.8 to 6.2], and high [≥ 6.2]) and postmortem aging (3 days and 21 days). This study especially fills a gap of knowledge on how would the aged intermediate pHu muscle, which is tougher, be differentiated in terms of lipids and metabolites that are likely to influence their sensory attributes.

II. MATERIALS AND METHODS

Pasture-finished Nellore bulls (*n* = 162) with 30-35 months of age, 4 to 6 permanent incisor teeth, were tracked since humanitarian slaughter until carcass processing in a commercial slaughterhouse. Nine *Longissimus lumborum* muscles were selected and classified based on the pHu after 72 h postmortem within pHu groups: 3 as Normal (< 5.8), 3 as Intermediate (5.8 to 6.2), and 3 as High (≥ 6.2) (Lomiwes et al., 2013). Two 2 cm steaks were cut from each muscle and were randomly assigned to two aging periods: 3 days [3-d] and 21 days [21-d]. They were individually vacuum-packed, totalizing 18 steaks (3 animals per each 3 pHu groups at each 2 aging periods). Postmortem aging was performed at 2 °C, and then the muscles were stored at -80 °C until analysis. Lipids were extracted for targeted lipid profiling, which was performed using discovery MRM-profiling methods and instrumentation (Xie et al., 2021). Mass spectrometry data were acquired (Antonelo et al., 2022), metabolites were extracted, and one-dimensional proton nuclear magnetic resonance (1D 1H NMR) spectra were acquired at 300 K using a Bruker Avance 14.1 T spectrometer (Bruker Corporation) at 600.13 MHz. Lipidome and metabolome data were uploaded to MetaboAnalyst 5.0, and data were Pareto-scaled. Hierarchical clustering heatmaps were performed. Pairwise comparisons were conducted: 1) High versus Normal, 2) High versus Intermediate, and 3) Intermediate versus Normal.

III. RESULTS AND DISCUSSION

The samples within the pHu groups tended to be clustered together at both postmortem aging periods, as exemplified for metabolites in the dendrograms shown in Figure 1. As major results, carnitines acylated with C12:0 and C14:0 fatty acids were identified as potential biomarkers of the intermediate pHu-muscle regardless the aging period. The normal pHu-muscle showed higher concentrations of metabolites of the glycogenolysis and glycolysis pathways, including glucose, mannose, and pyruvate. The high pHu-muscle was differentiated by the higher concentrations of fumarate (a metabolite of the

tricarboxylic acid cycle), formate (a metabolite of amino acid degradation), and acetate (produced by the action of reactive oxygen species on pyruvate). Interestingly, the concentration of arginine at early postmortem aging (3-d) may influence the previously reported improved tenderness in normal and high pHu-muscles at later aging (21-d).

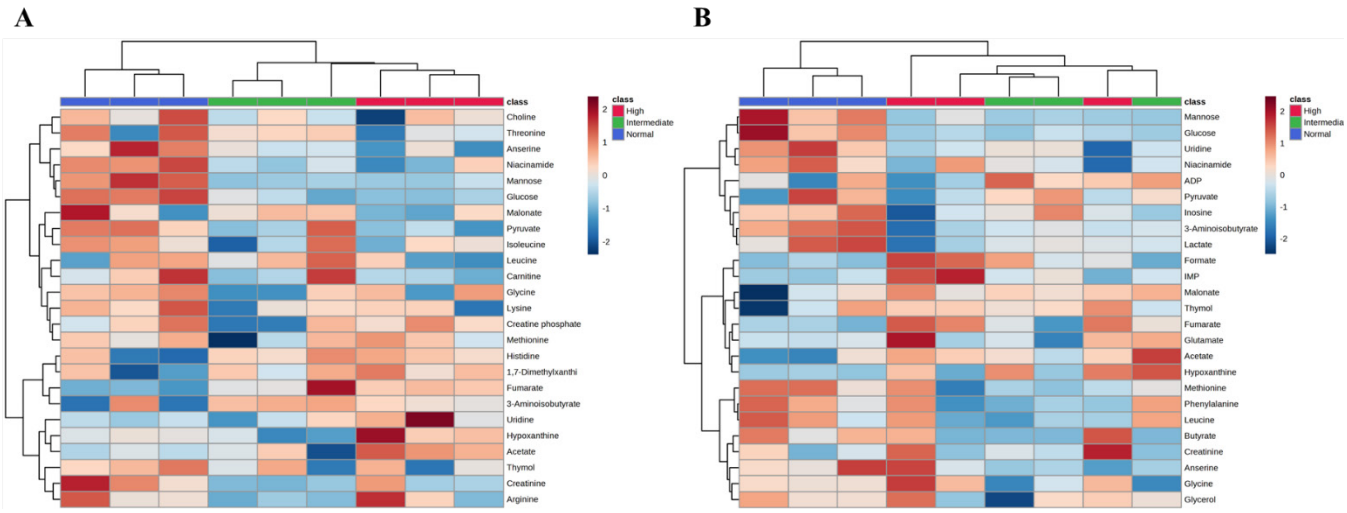

Figure 1. Hierarchical clustering heatmaps of the metabolome profiling of *Longissimus lumborum* muscles within ultimate pH groups (normal, intermediate, and high) at 3 days (A) and 21 days (B) of aging.

IV. CONCLUSION

Finding markers associated with biochemical changes that influence sensory attributes in meat is important for: a) using their levels to monitor aspects of meat production chain, such as antemortem stress level; and b) studying strategies to modulate the mechanisms that affect such markers levels to avoid meat defects. In our study, knowledge of postmortem biochemical changes of long-term aged beef within different pHu groups was raised based on preliminary data, which is essential to understand the mechanisms underpinning bull meat defects. Further studies should be encouraged to confirm our findings.

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EXPLORING THE BIOCHEMICAL BASIS FOR DARK BEEF DEVELOPMENT

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I. INTRODUCTION

The fresh meat quality attribute that consumers associate with the level of freshness and wholesomeness of beef is color. Products that do not meet the consumer's expectations for color cost the industry $3.73 billion annually, corresponding to 429.2 million pounds of beef discarded due to discoloration [1]. While extensive research has focused on beef color development in the past, the variability in beef color continues to plague the beef industry as 15% of retail beef does not meet the bright cherry-red color desired by consumers [2]. Beef color is influenced by myoglobin content, animal age, and the ultimate pH, which ranges from 5.5 to 5.8. Any deviations in ultimate pH are due mostly to pre-slaughter stress events that result in a condition known as dark-cutting beef. Yet, not all dark beef fits classical definitions of dark-cutting, this beef is known as atypically dark beef. Due to the vast differences in the mechanisms generating atypically and dark-cutting beef, there is a critical need to explore the biochemical basis for dark beef development and determine whether post-harvest processing strategies could reduce the incidence of dark beef in the marketplace.

II. MATERIALS AND METHODS

All procedures used were approved by the Virginia Tech Institutional Animal Care and Use Committee (#23-244). Twenty steers were randomly assigned into four treatments. Control (CON) cattle received saline injections 24 and 48 hours prior to harvest. In contrast, another group of cattle received two doses of 0.06 mg/kg of epinephrine at these same time points (T-2D). The remaining steers were assigned equally to either a single dose of epinephrine at 24 hours (T-24) or 48 hours (T-48) prior to harvest. At 1-hour post-harvest, carcasses were split and one side was subjected to electrical stimulation (ES, 300V, 15 Hz), while the other side served as a control. Samples from the liver and *Longissimus* muscle were collected pre-, post-, and 24 hours post-ES for metabolite and pH analyses. After 24 hours of chilling, color analyses were performed on 2.54 cm steaks collected from each side and aged for either 1, 7, or 14d. Data were analyzed as a completely randomized design in a 4 (epinephrine injections) x 2 (ES) factorial arrangement. The aged steak color was analyzed as repeated measurements. Differences were considered statistically significant when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

All epinephrine treated animals had reduced initial glycogen concentrations (Table 1). Lactate, G6P, and glucose didn't differ across treatments at 1 hr postmortem. Muscle from CON, T-24, and T-48 animals had a decrease in pH after ES treatment, however, no post-ES pH decline was observed for T-2D (Figure 1). Ultimate pH was affected by epinephrine injections as muscle from T-2D cattle resulted in carcasses with higher ultimate pH values. As expected, meat color was altered by epinephrine injections (Figure 2). Highest lean L* and a* values were noted in carcasses of CON animals whereas T-2D had the lowest values. Animals treated with one dose of epinephrine at either 48h or 24h pre-harvest showed intermediate color values. ES improved beef color, even from stressed, dark carcasses.

Table 1 – Effect of epinephrine injections on *Longissimus lumborum* metabolite concentrations at 1 hour postmortem.

Traits	Treatments				Pr > F
	Control	T-48	T-24	T-2D	
Lactate, µmol/g	5.86	5.47	3.11	3.83	0.7056
Glycogen, µmol/g	58.91a	35.31b	39.84b	27.61c	0.0001
G6P, µmol/g	5.86	5.47	3.11	3.83	0.3251
Glucose, µmol/g	3.09	2.51	2.02	1.71	0.4282
Liver Glyc. Pot., µmol/g	338.86	378.73	461.17	430.80	0.0109

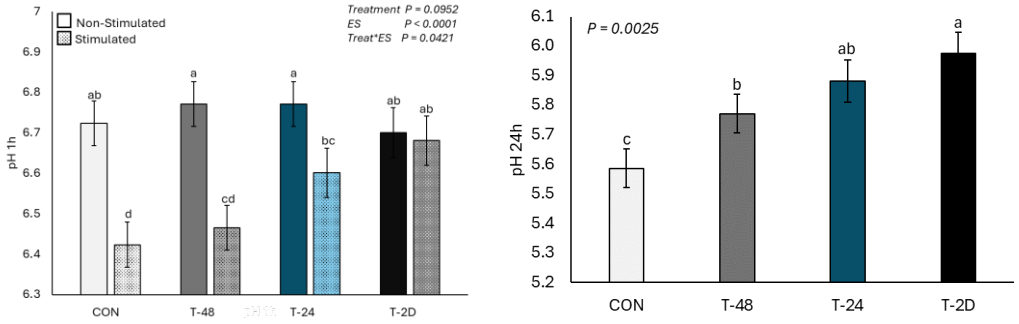

Figure 1. Effect of epinephrine injections and electrical stimulation on pH of *Longissimus thoracis*.

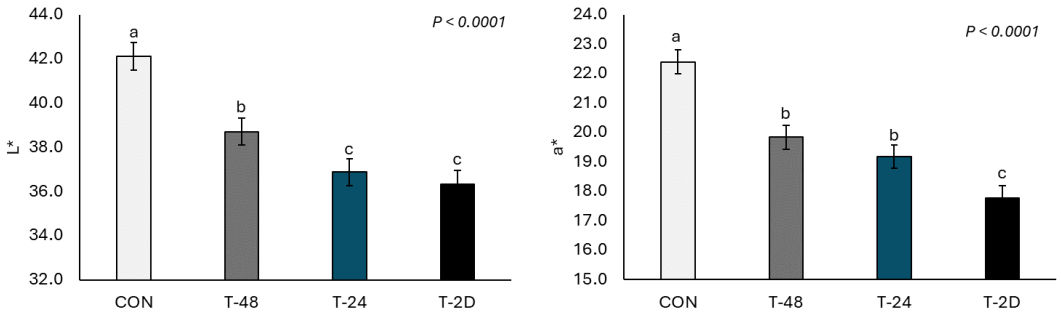

Figure 2. Effect of epinephrine injections and electrical stimulation on luminosity (L*, left) and redness (a*, right) of *Longissimus thoracis* over aging time.

IV. CONCLUSION

Two doses of epinephrine consistently lead to the production of dark-cutting beef. On the other hand, a single dose of epinephrine, regardless of the timing of the injection, produced the atypical dark beef phenotype. ES enhances the color of dark beef. Further research is underway to better understand the biochemical and physiological mechanisms driving the development of dark beef phenotypes.

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SESSION 5

Muscle biology & Meat quality

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RELATIONSHIPS BETWEEN TEXTURE AND WATER PROPERTY MEASUREMENTS IN RAW INTACT BROILER BREAST FILLETS WITH THE WOODEN BREAST CONDITION

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I. INTRODUCTION

The wooden breast (WB) condition significantly alters tactile characteristics in raw meat, with WB fillets (*Pectoralis major*) being much harder and more rigid [1,2]. Both subjective scoring and instrumental measurements are used to characterize the texture properties of raw WB fillets and have demonstrated texture differences [2]. Changes in connective tissue and collagen content, composition, and structure in muscle tissue are believed to be responsible for the tactile characteristics of WB meat [3]. Research has also demonstrated a relationship between muscle water and the WB condition in raw fillets [4,5]. We hypothesized that water properties may play a role in the altered texture properties of WB meat. The objective of this study was to investigate the relationship between water properties measured by low-field nuclear magnetic resonance (LF-NMR) and tactile characteristics in raw intact broiler fillets with the WB condition.

II. MATERIALS AND METHODS

Boneless skinless broiler breast fillets from 8-9 weeks old birds were collected from a commercial processing plant at approximately 3 h postmortem. Fillets (a total of 72 fillets, 24 for each group) were grouped using a 3-point scale for normal, moderate WB, and severe WB based on palpable hardness and rigidity. The Blunt Meullenet-Owens Razor Shear (BMORS) peak force was measured using a Texture Analyzer. A ^{1}H-NMR analyzer (LF 90II Proton-NMR, Bruker minispec, Billerica, MA, USA) was used to measure transverse relaxation (T_2) of raw fillets (after texture measurements) with the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [τ (90°-180° pulse separation) = 1 ms, 200 echoes, and 16 scans]. The decay curves were processed with the CONTIN regularization algorithm, resulting in the corresponding water properties, time constants (T), proportion (P), and normalized areas (A) for water populations 2b (fast time constant), 21 (medium time constant), and 22 (slow time constant). Spearman correlation coefficients were analyzed for relationships between WB scores and water property parameters (T_{2b}, T_{21}, T_{22}, A_{2b}, A_{21}, A_{22}, P_{2b}, P_{21}, and P_{22}) and Pearson correlation coefficients for relationships between BMORS force and water properties with SPSS software.

III. RESULTS AND DISCUSSION

In the fresh meat samples, there were three water populations observed with time constants (T_{2b}, T_{21}, and T_{22}) of 0.34-0.46, 44.2-67.2, and 156.2-301.9 ms, normalized abundances (A_{2b}, A_{21}, and A_{22}) of 1.3-5.2, 225.9-519.1, and 46.3-510.1 (area/100 g fresh meat weight), and relative proportions (P_{2b}, P_{21}, and P_{22}) of 0.20-1.43, 45.3-86.9, and 12.1-54.5% (data not shown). Table 1 shows both Spearman and Pearson correlations between water property parameters and subjective WB scores or BMORS measurements in raw broiler fillets. There were significant Spearman ($|r|$ = 0.46-0.78, $P < 0.001$) and Pearson ($|r|$ = 0.45-0.72, $P < 0.001$) correlations except for A_{21}. Strong and significant Spearman correlations ($|r| > 0.60$, $P < 0.001$) were found for T_{21}, A_{22}, P_{21} and P_{22} with P_{21} negatively ($r = -0.78$) and P_{22} positively ($r = 0.77$) associated with WB scores. However, a strong and signficant Pearson correlation was noted only between BMORS force and T_{21} ($r = 0.72$, $P < 0.001$). These results indicate that changes in the abundance of free water and in the mobility and proportion of

immobilized water are strongly associated with palpable hardness and rigidity of raw broiler fillets. However, only the change in mobility of immobilized water is strongly and positively associated with texture characteristics measured with the BMORS method. The Pearson r values for T_2 parameters T_{2b}, T_{21}, T_{22}, A_{2b}, A_{21}, and P_{2b} were similar to the Spearman r values (the differences were < 0.11); however, for T_2 parameters A_{22}, P_{22}, and P_{21}, the r values with BMORS were much smaller than those with WB scores (the differences is > |0.25|). A_{22}, P_{22}, and P_{21} were moderately (|r| = 0.49-0.50) correlated with BMORS peak force; however, they were strongly correlated with the WB scores (|r| = 0.74-0.78). Since BMORS measurements are the combination of shear and compression and the WB scores rely on both palpable hardness and rigidity, these results further suggest that the WB scores or the specific texture characteristics reflected by the subjective WB scores may be more influenced by the extra-myofibrillar water abundance. Simillarly, Pearce et al. [6] found that the decreased shear force was accompanied by decreases in T_{21} and P_{22} and increases in P_{21} in lamb M. Longissimus dorsi during postmortem aging and concluded that a high amount of intra-myofibrillar water and a low amount of extra-myofibrillar water may be associated with more tender meat.

Table 1. Correlation coefficients between water property measurements and subjective WB score (Spearman Correlation) or BMORS force (Pearson Correlation) in raw broiler breast fillets

Texture measurement	Time constant (ms)			Normalized area (area/100g)			Relative area (%)		
	T_{2b}	T_{21}	T_{22}	A_{2b}	A_{21}	A_{22}	P_{2b}	P_{21}	P_{22}
WB score[1]	0.46***	0.69***	0.54***	-0.55***	0.21	0.74***	-0.60***	-0.78***	0.77***
BMORS Force	0.45***	0.72***	0.46***	-0.47***	0.27	0.49***	-0.49***	-0.50***	0.50***

[1] WB score 1 = normal, 2 = moderate WB, and 3 = severe WB. BMORS = Blunt Meullenet-Owens Razor Shear. T_{2b} represents water with fast time constant (bound water), T_{21} represents water wtih medium time constant (immobilized water), and T_{22} represent water with slow time constant (free water). A_{2b}, A_{21}, and A_{22} represent normalized abundance of water with time constant T_{2b}, T_{21}, and T_{22}, respecitvely. P_{2b}, P_{21}, and P_{22} represent relative content or proportion of water with time constant T_{2b}, T_{21}, and T_{22}, respectively. * $P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$.

IV. CONCLUSION

1. Meat water properties, including population, mobility, and abundance, may be directly involved in the unique texture characteristics of the wooden breast meat or the severity of the wooden breast condition in broiler *Pectoralis major*.
2. Mobility of immobilized water (T_{21}), indicating the integrity of intramyofibrillar compartment, may be involved in texture hardness of raw WB meat.
3. Abundance of free water (A_{22}) may be involved in the rigidity texture attribute of raw WB meat.

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INFLUENCE OF GENDER STATUS ON INTRAMUSCULAR CONECTIVE TISSUE AND BEEF TENDERNESS IN DIFFERENT MUSCLES OF CROSSBRED ANGUS X NELLORE CATTLHE

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I. INTRODUCTION

The physiological differences between heifers, steers, and bulls can affect beef quality by modulating their growth and development [1]. Additionally, variation in beef quality can occur due to the type of muscle, as the proportions of different tissues vary among them. Intramuscular connective tissue supports muscle fibers and can influence muscle resistance, consequently affecting beef quality, especially in terms of tenderness [2]. It is suggested that gender status can affect the beef quality from different muscles of beef cattle due to differences in deposition of intramuscular connective tissue in these animals. Therefore, this study aimed to evaluate the influence of gender status and muscle type of crossbred Angus x Nelore cattle on intramuscular connective tissue and beef tenderness.

II. MATERIALS AND METHODS

Were used 102 F1 Angus x Nellore cattle, divided info bulls, steers and heifers. The animals were confined and kept under the same diet composed of 86% concentrate and 14% roughage for 150 days and were slaughtered at 16 months. The final mean weight was 488.9 ± 30.7 kg, 452.5 ± 24.9 kg, and 431.3 ± 26.3 kg for bulls, steers, and heifers, respectively. During boning, samples of the *Longissimus thoracis* (LT) and *Triceps brachii* (TB) muscles were collected and stored frozen at -18°C until analysis of shear force and quantification of collagen and its fractions. The shear force analysis was performed according to AMSA (2016) and the quantification of total, soluble and insoluble collagen was performed according to Woessner Junior (1961). The effects of gender status (bulls, steers and heifers) and muscle type (LT and TB) for shear force, levels of total, soluble and insoluble collagen were analyzed considering a completely randomized experimental design in a 3x2 factorial arrangement. Data was submitted to analysis of variance (ANOVA) using the MIXED procedure of the SAS® and the means of the results were compared using the Tukey test with a significance level of 5%.

III. RESULTS AND DISCUSSION

The results are presented on the Table 1. For the shear force, the meat of bulls presented higher results when compared to steers and heifers, which did not differ from each other (P = 0.001); and in relation to types of muscles, no differences were found between the LT and TB (P = 0.335). For the total collagen, there was no difference between the gender status (P = 0.448), and there was a difference between the muscles, where TB had a higher content (P <.000). For the soluble collagen, there was a difference for both factors, where bulls presented meat with higher collagen solubility content (P = 0.004), as well as muscle TB (P <.000). As for insoluble collagen, there was no difference between the gender status (P = 0.752), and TB had a higher content (P <.000). Bulls may have presented higher collagen solubility content due to the fact that they present a higher growth rate, which causes the intramuscular collagen to be continuously remodeled, so that the higher protein

turnover provides greater proportion of newly synthesized collagen, which is heat labile; however, bulls presented tougher meat. This finding can be explained by the fact that the animals were slaughtered young, suggesting that, in young cattle, other factors may be associated with differences in beef tenderness. Furthermore, in relation to the types of muscles, TB had a higher content of intramuscular collagen, which is justified by the fact that locomotion muscles normally present a higher content of this supporting tissue, however, the collagen did not affect the beef tenderness.

Table 1 – Shear force and and total, soluble and insoluble collagen levels on *Triceps brachii* (TB) and *Longissimus thoracis* (LT) muscles of crossbred Angus x Nelore cattle heifers, steers and bulls.

		Gender status					P-value		
Variable	Muscle	Heifers	Steers	Bulls	Mean	EPM	Gender status	Muscle	Gender status *Muscle
Shear Force (N)	TB	67.130	66.091	73.304	68.842	0.288	0.002	0.335	0.093
	LT	65.807	62.661	89.699	72.722	0.288			
	Mean	66.468B	64.376B	81.501A					
	EPM	0.353	0.353	0.353					
Collagen Total (mg/g)	TB	2.221	2.036	2.439	2.232a	0.079	0.137	<0.0001	0.151
	LT	1.217	1.547	1.538	1.434b	0.079			
	Mean	1.719	1.792	1.989					
	EPM	0.097	0.097	0.097					
Collagen Soluble (mg/g)	TB	0.373	0.379	0.487	0.413a	0.014	0.001	<0.0001	0.256
	LT	0.220	0.282	0.304	0.268b	0.014			
	Mean	0.296C	0.330B	0.395A					
	EPM	0.018	0.018	0.018					
Collagen Insoluble (mg/g)	TB	1.848	1.657	1.953	1.819a	0.067	0.318	<0.0001	0.139
	LT	0.997	1.266	1.234	1.166b	0.067			
	Mean	1.423	1.461	1.593					
	EPM	0.082	0.082	0.082					

TB: *Triceps brachii* muscle; LT: *Longissimus thoracis* muscle; EPM: Standard Error of the Mean. Means followed by the same uppercase letter in the rows and lowercase letters in the columns do not differ from each other using the Tukey test ($P \leq 0.05$).

IV. CONCLUSION

The intramuscular connective tissue was influenced by the gender status of the cattle and by the type of muscle, while the beef tenderness was influenced only by the gender status. Moreover, in this study, the constitution of intramuscular collagen does not seem to be related to variations in meat tenderness.

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PHYSICAL-CHEMICAL CHARACTERIZATION OF UNIQUE TEXTURE OF DRIED HORSE MACKEREL MEAT

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Ⅰ. INTRODUCTION

Dried horse mackerel was originally salted and dried to extend its shelf life. However, in recent years, due to health consciousness, the salt content has been lowered and the dried mackerel is distributed in the cold chain, but its preference remains popular with Japanese consumers. One reasons for consumer preference is its unique texture, along with its moderate saltiness and umami, which are enhanced by salt-drying. An appropriate texture for this product is described as “firm and elastic”. The texture of dried fish meat is thought to be affected by changes in the properties of the major muscle proteins, myosin and actin, as well as by water content [1]. However, the details of the mechanism behind the formation of the unique texture of recent low-salt dried fish have not been clarified.

Therefore, in this study, we dried salted horse mackerel meat and investigated changes in physical properties and muscle proteins.

Ⅱ. MATERIALS AND METHODS

Raw horse mackerel (*Trachurus japonicas*, body size 15-20 cm) from the sea near Japan was purchased, and the muscle was cut into small pieces, approximately 1 cm x 3 cm x 1 cm, and immersed in 1 L of 1.5 M NaCl/20 mM Tris-acetate (pH 7.0) to be salted at 4°C for 20 min. This was dried for 0-4 h in an air circulation incubator at 30°C. The salt content and moisture content of the sample after drying were measured. In addition, the breaking strength of each sample was measured using a creep meter (YAMADEN, RE-33005s) equipped with a cylindrical plunger with a diameter of 2 mm. The Mg^{2+}- and Ca^{2+}-ATPase activities of the sample homogenate were measured to estimate the degree of denaturation of actin and myosin [2]. Furthermore, myofibrils prepared from the sample were subjected to SDS-PAGE using a 6 M urea-2% gel [3] to examine the generation of myosin polymer.

Ⅲ. RESULTS AND DISCUSSION

As a result of salting the horse mackerel meat sample for 20 minutes, the salt concentration was around 0.3 M. This is approximately the same concentration as the recent dried horse mackerel described in Japanese government database [4]. As a result of drying it, the moisture content reached 68% after 2 h of drying, being almost the same as commercial dried mackerel products. The breaking strength increased with drying for 0-4 h. As shown in

Figure 1, the Mg^{2+}- and Ca^{2+}-ATPase activities of the homogenate prepared from dried

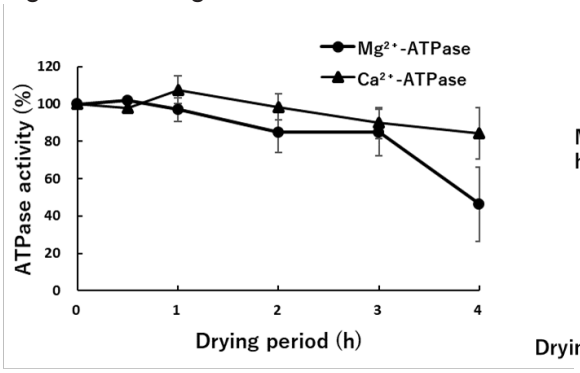

Figure 1. Changes of Mg^{2+} and Ca^{2+} -ATPase activities during drying process

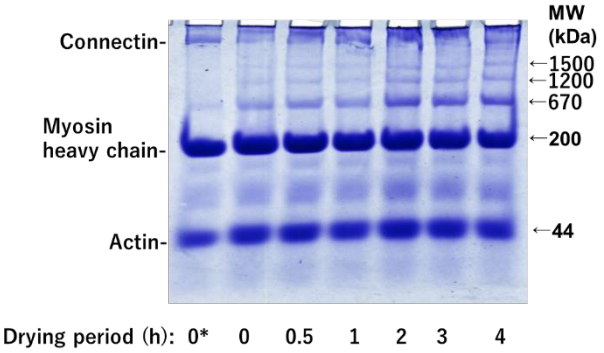

Figure 2. Urea-SDS-PAGE pattern of myofibrils prepared from dried horse mackerel meat. * control (untreated meat)

horse mackerel meat decreased during 4 h-drying, but the Mg^{2+}-ATPase activity decreased faster than the Ca^{2+}-ATPase activity. Therefore, it was suggested that actin was denatured earlier than myosin. Being similar with a heating gel of a mixture of actin and myosin [5], the increase of myosin/actin ratio induced by faster denaturation of actin than myosin might increase breaking strength during drying. Furthermore, three new bands (MW 1500, 1200, 670 kDa) presumable to be polymer of myosin heavy chain were detected by urea-SDS-PAGE of the myofibrils, which increased with drying (Figure 2), similarly with dried walleye pollack meat [6]. Therefore, the newly formed bonds between myosin molecules indicated by the appearance of such polymers could cause an increase in breaking strength due to drying.

Ⅳ. CONCLUSION

During drying of salted horse mackerel meat, the breaking strength increased. The binding between denatured actin and myosin presumably promoted by the faster denaturation of actin than myosin, and the formation of polymers of myosin heavy chain would strengthen the meat structures. These are probably responsible for the unique texture of dried horse mackerel meat.

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Conflicts of Interest: M. Matsuishi is a board member of Starzen Co., Ltd.

Comparison of quality characteristics according to the carcass chilling conditions of goat

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I. INTRODUCTION

The black goat (*Capra hircus*) is a domesticated wild goat belonging to the Bovidae and is characterized by its black fur [1]. Due to its distinctive odor, the black goat (*Capra hircus*) is primarily utilized for medicinal purposes rather than as a meat source, particularly in South Korea [2]. However, in South Korea, as its potential as a low-fat, high-protein meat source gains recognition, the consumption pattern of black goat is gradually shifting towards meat. With the increasing consumption of black goat meat, various studies are being conducted to enhance its suitability as an edible meat. However, there is a lack of research on the edible meat characteristics of goats regarding to carcass chilling. Carcass chilling is one of the crucial aspects influencing the final quality of goat meat as it impacts the rate of decrease in muscle temperature and pH [3]. Therefore, this study analyzed the physicochemical quality characteristics of black goat meat under different chilling temperatures (-3 or +4℃) to provide fundamental data for further research on goat meat.

II. MATERIALS AND METHODS

The 10 goats were slaughtered at the slaughterhouse of the National Institute of Animal Science. Subsequently, 5 goats each were chillled at temperature of -3℃ and +4℃, respectively, for 24 hours.

pH values and carcass temperature were monitored at 5 minute intervals for 24 hours post slaughter using a pH meter and thermometer, respectively, in both the loin and rump. In meat quality analysis, including meat color, water holding capacity, and shear force, samples were taken from the loin after 24 hours of slaughter. Meat color analysis was conducted using a chroma meter (CR-400, Konica Minolta, Sensing, INC.) to assess lightness, redness, and yellowness. Water holding capacity was determined according to the method described by Fischer et al (1976). Shear force measurements were performed using a Warner-Bratzler shear meter, with samples cut perpendicular to the direction of muscle fibers. Microbial testing was conducted on live animals prior to slaughter and on meat 24 hours thereafter. Swab samples were collected from 6 representative locations (3 locations per side x2 sides: shoulder, hip (cranial-cauda midline), and ham) of each animal.

Statistical analyses were carried out using Duncan's multiple range tests with the SAS program. Results with a p-value less than 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

Rate of extent of pH decline are known to affect the development of meat quality characteristics such as color and tenderness [4]. In this study, the muscle pH gradually decreased from about 6.4 to 5.7 during chilling at –3℃, and dropped to 5.4 during chilling at +4℃. Similar results have been reported in cattle depending on the chilling temperature [5]. Studies suggest that low-temperature chilling delays the pH reduction rate. Meat color is an important characteristic affecting consumer perception of product quality [6]. The characteristic color of goat meat has not been firmly established, there is a perception that it is darker compared to other types of red meat. In this study, the average values of L*(lightness), a*(redness), and b*(yellowness) ranged from 36.49 to 37.47, 16.37 to 17.82, and 4.58 to 5.44, respectively, with significant differences observed only in the a* value depending on the chilling temperature. Additionally, the tenderness of goat meat was influenced by the chilling temperature, with shear force values being lower for goat meat chilled at –3℃ compared to +4℃.

Furthermore, the water holding capacity in the –3℃ chilling treatment group are similar to the findings reported by Bouton et al(1983), who suggested that as water holding capacity decreases, shear force increases. Chilling temperature did not affect the microbial count at the surface of carcasses (data not shown).

Table 1 – Physico-chemical characteristics of goat meat

Trait		Chilling conditions	
		-3℃	+4 ℃
Color	CIE L*	37.42 ± 1.21	36.49 ± 2.85
	CIE a*	16.37 ±0.58^{b}	17.82 ± 0.86^{a}
	CIE b*	4.58 ± 0.32	5.44 ± 0.87
WHC (%)		72.53 ± 1.25^{b}	73.77 ± 1.10^{a}
Shear force (kgf)		4.07 ± 0.57^{b}	5.24 ± 0.62^{a}

a-b Mean ± SD in the same rows with different letters are significantly different ($p<0.05$).

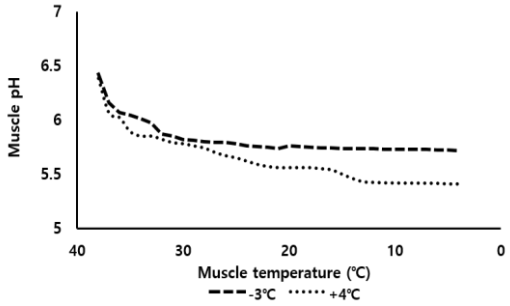

Figure 1. The pH and temperature of muscle

IV. CONCLUSION

In this study, the physicochemical properties of goat meat according to the chilling conditions were investigated. These finding suggest that the chilling temperature of carcass may have an effect on water-holding capacity, meat color and shear force in goat meat.

ACKNOWLEDGEMENTS

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Effects of dry ageing in bag and thermal processing on the fatty acid profile of Serpentina chevon

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I. INTRODUCTION

Goat meat is recognised as a high quality product, for its leanness, low cholesterol and saturated fatty acid content [1], while rich in protein of high nutritional value, leading to strong market value. However, older animals produce less tender meat, often used as by-products, due to its devaluation and consequent sale at lower prices [2, 3]. In Portugal, the consumption of goat meat is a deeply rooted tradition, where *Serpentina*, a native goat breed has been granted IGP status ("Protected Geographical Indication") [4]. In-bag dry-ageing is a meat ageing technique commonly applied to beef, in which cuts of meat are packed in bags that are highly permeable to water vapour and then stored in refrigerated environments, at temperatures between 1 and 4 ºC for a limited period, in order to produce more tender, tasty and succulent meats [5, 6]. Therefore, the aim of this study was to evaluate the effects of dry ageing in bag and thermal processing, on the fatty acid composition of the *Longissimus dorsi* muscle of 5 female *Serpentina* goats, slaughtered between 8 and 12 years old.

II. MATERIALS AND METHODS

Five *Serpentina* females from the same farm were used in this study. The animals were slaughtered between 8 and 12 years of age, with an average final carcass weight of 28.4 ± 4.4 kg, in accordance with European Commission regulations. On the second day *post-mortem*, *longissimus thoracis et lumborum* muscle (LTL) was cut from both sides of the carcass and vacuum-packed. On the third day *post-mortem*, the left *longissimus dorsi* was packed in bags with an O_2 transmission rate of 24 $cm^3/(m^2$ 24 h) at 23 ºC and 0% RH; a CO_2 transmission rate of 78 $cm^3/(m^2$ 24 h) at 23 ºC and 0% RH; and a water vapour transmission rate of 44 $g/(m^2$ 24 h) at 38 ºC and 100% RH (LID540X, Cryovac). Afterwards, they were allowed to age for 46 days at 2±2 ºC with a relative humidity of 60-90%, in the dark, with unfiltered air and protected from UV light. From the right side of the LTL muscle, 200g were removed for analyses and the remainder was frozen at -80 ºC to serve as a reference of unaged meat. Both aged and unaged meat were cooked in a dry oven at 150 ºC until the internal temperature reached 68 ºC. The fatty acid composition was determined in all samples from the lipid extract of the freeze-dried meat, by direct transesterification with 0.5 M sodium methoxide in methanol, at 50 ºC for 30 minutes, followed by reaction with 1.25 M HCl in methanol, at 80 ºC for 15 minutes. The fatty acids were then extracted using hexane and the excess solvent was removed by a nitrogen stream at 37 ºC. The fatty acids were identified by comparing the gas chromatography retention times with commercial standards and published chromatographs [7]. The data was analysed in SAS following a 2x2 factorial design with PROC MIXED procedure, with ageing and cooking as fixed effects, and their interaction. Significant interactions were indicated when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

The fatty acid composition of the meat is shown in Table 1. A significant interaction between ageing and thermal processing on the total fatty acid content (TFA) was observed. It should be noted that although the total fatty acid content showed a significant increase in aged meat

(126 mg/g) when compared to non aged meat (63 mg/g), probably due to the effect of ageing process, after heat treatment, no significant differences were observed between aged and unged cooked samples. Saturated fatty acids (SFA) proved to be the most abundant category, followed by monounsaturated acids (MUFA), and lastly, polyunsaturated fatty acids (PUFA), with no significant interaction effect (ageing x cooking). Myristic (14:0) and arachidonic (20:4n-6) fatty acids showed a significant interaction between ageing and thermal processing. Palmitic acid (16:0) stood out as the most predominant SFA, while oleic acid (18:1c9) was the most abundant MUFA, and linoleic acid (18:2n-6) was the most abundant PUFA. Oleic acid (18:1c9), with contents between 38.23 and 39.74 g/100g of total fatty acids, was the major MUFA, contributing with about 86% of the total MUFA. There was no significant interaction between ageing x thermal processing and no main effect of thermal processing for 16:0 (P > 0.105). Ageing resulted in an increase in 16:0 (P = 0.006) and a decrease in 18:2n-6, 18:3n-3 and 20:5n-3 (P < 0.05) as shown in Table 1. These results are in line with the changes observed in SFA and PUFA.

Table 1: Total fatty acid content (mg/g DM) and fatty acid profile (g/100g total acids) of unaged and dry-aged meat samples (raw and cooked).

Traits	Control		Dry-Aged		SEM	Effects		
	Raw	Cooked	Raw	Cooked		Dry-Aged	Cooked	Dry-aged*Cooked
TFA	63^{b}	100^{ab}	126^{a}	81^{ab}	11.6	0.084	0.781	<0.001
12:0	0.07	0.09	0.08	0.07	0.006	0.975	0.332	0.053
14:0	1.90^{b}	2.25^{a}	2.17^{ab}	2.17^{ab}	0.071	0.197	0.029	0.031
16:0	24.15	23.01	24.64	24.57	0.304	0.006	0.073	0.105
16:1*c*9	1.65	2.45	1.72	2.14	0.217	0.589	0.016	0.409
18:0	18.94	18.27	21.91	19.13	0.937	0.063	0.091	0.279
18:1*c*9	38.86	39.74	38.23	39.69	1.384	0.809	0.416	0.839
18:2n-6	3.45	2.99	2.28	2.42	0.235	0.003	0.504	0.224
20:0	0.07	0.09	0.10	0.08	0.010	0.377	0.864	0.132
18:3n-3	1.11	0.96	0.82	0.86	0.084	0.037	0.573	0.295
20:4n-6	1.85^{a}	1.28^{ab}	0.79^{b}	1.16^{ab}	0.207	0.015	0.648	0.041
20:5n-3	0.71	0.46	0.29	0.45	0.097	0.048	0.658	0.057
22:5n-3	0.90	0.74	0.48	0.63	0.138	0.082	0.989	0.282
∑SFA	47.28	46.55	51.34	48.37	1.152	0.025	0.134	0.351
∑*cis*-MUFA	42.47	44.84	41.84	43.93	1.510	0.620	0.166	0.929
∑*trans*-MUFA	1.24	1.31	1.50	1.36	0.128	0.242	0.796	0.435
∑PUFA	9.01	7.31	5.32	6.34	0.84	0.017	0.689	0.132

Means followed by different superscripts within a column differ significantly at $P \leq 0.05$; SEM: standard error of the mean.

The increase in SFA and decrease in PUFA with ageing is likely due to lipid peroxidation during the long ageing period. Lean meat is particularly rich in phospholipids, which contain membrane long-chain PUFA, motably 20:4n-6. On the other hand, meat with a high intermuscular and subcutaneous fat content are comprised mostly of triacylglycerols rich in SFA and MUFA and a lower content of long-chain PUFA [7].

IV. CONCLUSION

Serpentina chevon aged for 46 days and thermally processed presented a fatty acid profile predominantly composed of 18:1c9, 16:0, 18:0, 18:2n-6 and 20:4n-6. Ageing and thermal processing showed significant impacts on 14:0 and 20:4n-6 fatty acids, highlighting the influence of these processes on the lipid composition of meat.

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Microbiological status of *Serpentina* chevon dry aged in bag

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I. INTRODUCTION

Dry ageing in bag is a *post-mortem* process for increasing the quality of meats, with distinctive flavour, texture, and tenderness characteristics [1], [2]. This process involves the use of packaging bags with a high water vapour transmission rate [1]. Goat meat plays a significant role in the Portuguese diet, typically consumed from fresh meat. During ageing it is essential to rigorously monitor the process conditions to promote the development of desirable sensory characteristics and control the growth of undesirable microorganisms, especially pathogenic bacteria. Previous studies reveal challenges concerning the microbiological safety of aged meat partly due to the absence of specific regulations, further complicating risk assessment and safety assurance [3]. Several studies have highlighted the connection between meat ageing and the potential emergence of microorganisms that cause foodborne illnesses [3], [4], [5], [6]. Therefore, the main objective of this study was to assess the microbiological safety of goat meat dry aged in bag for 46 days, as well as the effects of thermal processing.

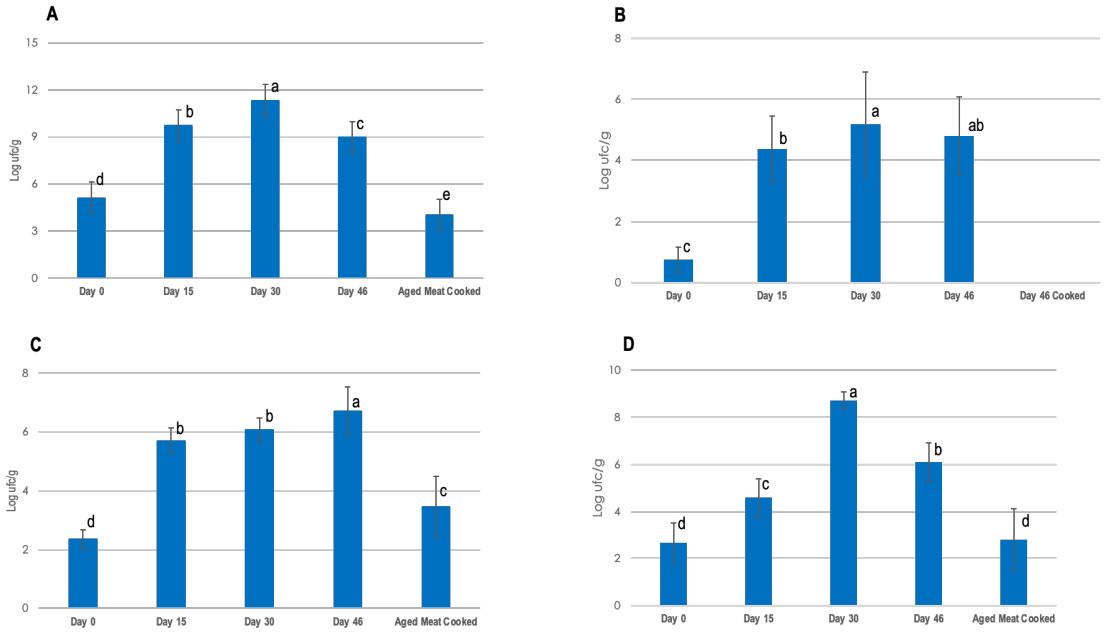
II. MATERIALS AND METHODS

Five female *Serpentina* goats from the same farm were slaughtered in a commercial abattoir in accordance with the European Commission's regulatory guidelines. The animals were between 8 and 12 years old, with an average final carcass weight of 28.4±4.4kg. On the second day *post-mortem*, the *longissimus thoracis et lumborum* muscle (LTL) was cut from both sides of the carcass and vacuum-packed. On the third day *post-mortem*, the left LTL muscle was packed in bags with OTR permeability (at 23 ºC and 0% RH) of 24 $cm^3/m^2/24$ h; CO_2 permeability (at 23 ºC and 0% RH) of 78 $cm^3/m^2/24$ h and MVTR permeability (at 38 ºC and 100% RH) of 44 $g/m^2/24$ h (LID540X, Cryovac®, and allowed to age for 46 days at 2 ± 2 ºC with relative humidity of 60-90%, in the dark, with unfiltered air and protected from UV light. Samples from the right-side LTL muscle were used for microbiological analyses throughout the ageing period (days 0, 15 and 30). This meat was then cooked in a dry oven at 150 ºC until the internal temperature reached 68 ºC. All the microbiological analyses were processed in different culture media and carried out in accordance with the corresponding ISO standards for the following parameters: Total count bacteria, *Escherichia coli* (*E.coli*) count, *Salmonella* spp., *Pseudomonas* spp. count, *Bacillus cereus* count, Mould and Yeast count, and Lactic Acid Bacteria (LAB) count. Microbial results were reported as present or absent for *Salmonella* spp, while counts were expressed in colony-forming units per gram and transformed into log colony-forming units per gram (log_{10} cfu/g) before statistical analysis. The data was analysed in the *RStudio* statistical software (version 4.2.2) using the non-parametric Kruskal Wallis test ($P \leq 0.05$).

III. RESULTS AND DISCUSSION

The microbiological results are shown in Figure 1 for each microorganism detected in this study. The presence of *Salmonella* spp. was not observed, nor was the proliferation of *Bacillus cereus* and *Pseudomonas* spp.. On the other hand, *E.coli*, moulds and yeasts, LAB, and total aerobes, were influenced by ageing and thermal processing. It was found that the highest counts of microorganisms were obtained for total aerobes and LAB. In particular, higher microbial counts were recorded on the 30th day of ageing compared to days 0, 15 and 46 for total aerobes, *E.coli* and LAB, unlike moulds and yeasts which showed higher counts on day 46 (Figure 1). Before ageing, the total bacterial count was

5.1 log cfu/g, the *E.coli* count was 0.75 log cfu/g, moulds and yeasts 2.3 log cfu/g and LAB 2.6 log cfu/g. During the ageing period, exponential microbial growth was observed until the 30th day of ageing, followed by a subsequent decline. We highlight that elevated counts during ageing align with the fact that microbiological analyses were conducted on untrimmed meat. The contamination levels obtained reflected the maximum microbial load achievable during ageing, consistent with previous findings indicating high levels of microbial contamination on the surface of aged meat [3]. Importantly, thermal processing (cooking) effectively reduced the microbial load to values below the detection limit (<1 log cfu/g) for *E.coli* count.



1. Figure 1: Microbiological profile of *Serpentina* chevon (A–Total bacteria counts; B–E.coli; C–Moulds and Yeasts; D–5rrrrrhb7yhu++º’’’’’’’’’’9,,,,p LAB).

The reduction in the total bacterial count on the 46th day of the ageing process can partially be attributed to the competition between microorganisms during ageing process. However, considering the high microbial load of the meat before the beginning of the ageing process and that ageing only began on day 3 post mortem, is it possible that hygienic conditions during slaughter and storage may have led to carcass contamination. Moreover, in a cross-sectional study related with aged products, it was reported that the current knowledge of the microbiological quality and safety of aged meat is still limited, suggesting the need for additional studies to evaluate the safety of these products [7].

IV. CONCLUSION

The results showed that ageing time led to increasing counts of *E.coli*, total aerobes, LAB and moulds and yeast. Thermal processing reduced the microbial load, being particularly effective for *E.coli*. These results highlight the importance of microbiological control during meat ageing.

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CHEMICAL COMPOSITION OF ORGANIC FREE-RANGE ROOSTER MEAT AS AFFECTED BY THE DIETARY SUPPLEMENTATION WITH AGRO-INDUSTRIAL BY-PRODUCTS AND FLAX SEED

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I. INTRODUCTION

Poultry production systems based on free-range breeding of birds are being implemented as an alternative to large-scale conventional breeding operations [1]. On the other hand, the short cycle of poultry allows the use of agro-industrial by-products as feed, which can be transformed into edible meat and eggs [2]. In addition, the inclusion of these supplements in the diet of birds may improve their health and meat quality by increasing the intramuscular content of healthy fats, such as omega-3 (ω-3) and omega-6 (ω-6) polyunsaturated fatty acids (PUFAs) [3]. Taking into account the above-mentioned points, we proposed to study the influence of different fattening diets on the chemical composition of organic free-range rooster meat using various agro-industrial by-products, such as beer bagasse (BB) and olive pomace (OP), and flax seed (FS), an underused raw material in animal feeding in Spain.

II. MATERIALS AND METHODS

II.I. Animals and feeding

All roosters used in this study were males. These were raised for 3 months and then underwent fattening diet for the next 4 months in semi-freedom pens until slaughter. In this last stage, the birds were classified according to their diet into the following batches: control (CO), BB, OP, and FS. The diet of the CO batch was based exclusively on corn, wheat, and peas. This mixture was added with 5% (w/w) of BB, OP, and FS in each of the other batches, as appropriate.

II.II Sampling

After the first 24 h of sacrifice, the breasts of 10 birds per batch, of a total of 40, were randomly sampled. Proximal analysis was carried out on each of the breasts by determining the moisture, protein, intramuscular fat, and ash content. In addition, the fatty acids were also identified and quantified following the procedure described by Dominguez et al. [4].

II.III Statistical analysis

The detection of significant differences among the different batches was carried out through a one-way analysis of variance (ANOVA) using the IBM SPSS Statistics 23.0 program (IBM Corporation, Somers, NY, USA). Least square means were separated using Duncan's *post hoc* test (significance level $P<0.05$).

III. RESULTS AND DISCUSSION

The results hardly showed differences in the proximate composition of the breasts (Table 1). The different fattening diets did not affect the percentage of fat and protein. However, the Duncan's multiple range test displayed significant differences ($P<0.05$) among batches for the moisture and ash. Specifically, higher moisture was observed in the CO batch and a lower ash content in the BB batch. Similar values were found in breasts of broilers fed PUFA-rich food by-products [5]. The variation in the rooster's diet resulted in different fatty acid profiles. The BB batch showed a higher PUFA content than the CO batch, and ω-3 fatty acids were significantly higher when BB, OP, and FS were used during the fattening phase of the birds (Table 2). These compounds are highly prized due to their reported potential health effects [6]. However, the increase in ω-3 PUFAs was not

reflected in the ratio of ω-6:ω-3 fatty acids, which was above the value recommended for human nutrition (4:1).

Table 1 – Influence of different fattening diets using agro-industrial by-products and flax seed on the chemical composition of organic free-range rooster breasts (*n* = 10).

g/100 g of meat	Batch				SEM	Sig.
	CO	BB	OP	FS		
Moisture	73.28 ± 0.64^{a}	72.40 ± 0.85^{b}	72.4 ± 0.87^{b}	72.84 ± 0.91^{a,b}	0.14	ns
protein	24.66 ± 0.32	25.01 ± 0.92	24.81 ± 0.73	24.99 ± 0.59	0.12	ns
Fat	0.94 ± 0.56	1.07 ± 1.23	1.46 ± 1.32	1.13 ± .93	1.16	ns
Ash	1.18 ± 0.04^{a,b}	1.14 ± 0.06^{a}	1.18 ± 0.05^{b}	1.19 ± 0.03^{b}	0.01	ns

CO: control; BB: beer bagasse; OP: olive pomace; FS: flax seed; SEM: standard error of mean; Sig.: significance (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; ns, not significant). $^{a-c}$Means in the same row not followed by a common superscript letter are significantly different (*P*<0.05; Duncan's test).

Table 2 – Influence of different fattening diets using agro-industrial by-products and flax seed on the fatty acid profile of organic free-range rooster breasts (*n* = 10).

mg/100 g of meat	Batch				SEM	Sig.
	CO	BB	OP	FS		
SFAs	402.19 ± 172.09	687.34 ± 446.51	538.32 ± 310.77	473.59 ± 223.10	49.49	ns
MUFAs	487.95 ± 234.16^{a}	997.13 ± 706.86^{b}	725.68 ± 477.05^{a,b}	634.97 ± 371.42^{a,b}	78.68	ns
PUFAs	205.42 ± 49.62^{a}	366.24 ± 175.46^{b}	289.83 ± 108.11^{a,b}	275.60 ± 83.49^{a,b}	19.57	*
ω-3 FAs	23.70 ± 3.66^{a}	42.09 ± 12.56^{b}	33.69 ± 4.37^{c}	34.69 ± 8.71^{b,c}	1.62	***
ω-6 FAs	178.72 ± 46.05^{a}	319.69 ± 163.22^{b}	252.68 ± 104.25^{a,b}	237.48 ± 74.51^{a,b}	18.03	*
ω-6:ω-3	7.5 ± 1.3	7.42 ± 2.14	7.43 ± 2.45	6.82 ± 1.17	0.28	ns

SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; ω-3 FAs: omega-3 fatty acids; ω-6 FAs: omega-6 fatty acids; CO: control; BB: beer bagasse; OP: olive pomace; FS: flax seed; SEM: standard error of mean; Sig.: significance (*, *P*<0.05; **, *P*<0.01; ***, *P*<0.001; ns, not significant). $^{a-c}$Means in the same row not followed by a common superscript letter are significantly different (*P*<0.05; Duncan's test).

IV. CONCLUSION

Feeding organic free-range roosters with BB, OP, and FS barely produced significant changes in the proximal composition of the breasts. However, remarkable differences were observed in the amount of ω-3 PUFAs, suggesting the storage of these compounds in the meat. This finding might open an interesting avenue for experimentation in poultry feeding.

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BRAZILIAN BEEF: A FOCUS ON TENDERNESS AND SARCOMERE LENGTH

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I. INTRODUCTION

With the world's second-largest cattle herd, Brazil assumes a significant role in global meat production, achieving a volume of 10.27 million tons in 2022 [1]. Owing to the country's extensive territorial diversity, this production is influenced by geographic conditions, genetics, production systems, technology, and market demand, leading to variations in meat quality attributes such as color, flavor, and tenderness, crucial for consumer satisfaction. Tenderness is closely associated with ante-mortem factors (e.g., breed, sex, maturity, and feeding) and post-mortem factors (e.g., cooling and electrical stimulation) [2]. These factors, in turn, can impact sarcomere length, fundamental structural unit of the myofibril. Therefore, the present study aims to investigate the quality of Brazilian beef produced in various slaughterhouses across the country, focusing specifically on the relationship between tenderness and sarcomere length.

II. MATERIALS AND METHODS

Samples (n=111; Zebu breeds) of the *Longissimus thoracis* muscle were collected from three slaughterhouses in Brazil (São Paulo, Bahia, and Rondônia). From each facility, five different pens were selected, representing distinct groups of animals (with 5 to 8 samples each; see Table 1). All samples were frozen 48 hours after slaughter. The pH was measured following the protocol outlined by Ramos and Gomide, 2017 [3]. Fat content (%) was determined using near-infrared spectroscopy (FoodScan 2 PRO, Hillerød, Denmark) [4]. Sarcomere length (µm) was measured via laser diffraction, following the methodology described by Battaglia et al., 2020 [5]. Instrumental tenderness (kg; Warner-Bratzler Shear Force) was determined following the guidelines provided by AMSA, 1995 [6]. Analyses were conducted in triplicate, and the data underwent one-way analysis of variance (ANOVA) followed by Tukey's test ($p<0.05$) using Statistica® version 10.0. Additionally, the Pearson Correlation coefficient was calculated for sarcomere length and tenderness.

III. RESULTS AND DISCUSSION

The samples exhibited final pH values ranging from 5.4 to 6.5. However, those with pH ≥ 5.9, indicative of DFD (dark, firm, and dry) characteristics, were excluded from this study to mitigate their impact on tenderness and ensure result consistency. Table 1 illustrates the pen characteristics alongside the results of fat content, sarcomere length, and instrumental tenderness of the samples.

Table 1. Means ± standard error of beef quality parameters from cattle at three Brazilian slaughterhouses.

	Sex	Teeth (Age)	HCW (kg)	EE	Fat (%)	SL (µm)	WBSF (kg)
Slaugterhouse A							
Pen 1, n=6	Intact male	2	342	Yes	2,97 ± 0,10 ^{b}	1,77 ± 0,04 ^{a}	4,87 ± 0,30 ^{a}
Pen 2, n=8	Intact male	2	290	Yes	3,96 ± 0,27 ^{a}	1,73 ± 0,05 ^{a}	6,14 ± 0,49 ^{a}
Pen 3, n=7	Intact male	2	316	Yes	2,78 ± 0,16 ^{b}	1,71 ± 0,03 ^{a}	5,74 ± 0,22 ^{a}
Pen 4; n=6	Intact male	2	342	Yes	3,01 ± 0,20 ^{b}	1,79 ± 0,03 ^{a}	4,90 ± 0,17 ^{a}
Pen 5; n=8	Intact male	2	252	Yes	3,55 ± 0,15 ^{ab}	1,73 ± 0,05 ^{a}	6,01 ± 0,50 ^{a}
Mean	-	-	-	-	3,28 ± 0,11 ^{A}	1,75 ± 0,02 ^{A}	5,56 ± 0,18 ^{C}
Slaugterhouse B							
Pen 6; n=5	Intact male	6	296	No	1,70 ± 0,14 ^{b}	1,63 ± 0,06 ^{ab}	6,98 ± 0,75 ^{ab}
Pen 7; n=8	Intact male	4	314	No	3,24 ± 0,19 ^{ab}	1,66 ± 0,01 ^{ab}	5,59 ± 0,31 ^{ab}
Pen 8; n=8	Female	8	252	No	4,18 ± 0,51 ^{a}	1,57 ± 0,03 ^{b}	6,91 ± 0,63 ^{ab}
Pen 9; n=8	Intact male	4	294	No	4,24 ± 0,69 ^{a}	1,71 ± 0,01 ^{a}	5,10 ± 0,24 b

Pen 10; n=7	Intact male	2	290	No	2,26 ± 0,15 [b]	1,57 ± 0,05 [ab]	7,59 ± 0,71 [a]
Mean	-	-	-	-	3,27 ± 0,25 [A]	1,63 ± 0,02 [B]	6,36 ± 0,28 [B]
Slaugterhouse C							
Pen 11; n=8	Female	8	160	Yes	3,10 ± 2,29 [a]	1,56 ± 0,05 [a]	6,31 ± 0,30 [a]
Pen 12; n=8	Intact male	4	300	Yes	1,71 ± 0,10 [a]	1,48 ± 0,04 [a]	8,03 ± 0,58 [a]
Pen 13; n=7	Female	8	196	Yes	2,39 ± 0,11 [a]	1,58 ± 0,03 [a]	6,24 ± 0,25 [a]
Pen 14; n=8	Female	8	208	Yes	2,95 ± 0,20 [a]	1,45 ± 0,05 [a]	7,38 ± 0,52 [a]
Pen 15; n=7	Female	2	170	Yes	3,00 ± 0,29 [a]	1,53 ± 0,10 [a]	7,67 ± 0,48 [a]
Mean	-	-	-	-	3,09 ± 0,50 [A]	1,52 ± 0,03 [C]	7,13 ± 0,23 [A]

EE: Electrical Stimulation; SL: Sarcomere Length; HCW: Hot Carcass Weight. Different letters within the same column indicate a significant difference between samples as determined by the Tukey test ($p < 0.05$). Lowercase letters denote statistical comparisons among pens, while uppercase letters denote comparisons between slaughterhouses.

The average carcass weight was 269 kg. While fat content varied among some pens, no differences were observed between units. Sarcomere length ranged from 1.21 to 2.05 µm, with notable emphasis on pens from unit A, displaying the highest values. This could be attributed to the larger carcass weight coupled with the application of electrical stimulation, which accelerates the decline in muscle pH, thereby preventing excessive sarcomere shortening [5]. Regarding instrumental tenderness, shear force exhibited a range of 3.6 to 10.4 kg, with only approximately 15% of samples classified as very tender (> 3.0 kg) or tender (4.0 – 4.4 kg). Pens from unit A featured tender meat, possibly owing to the younger age of the animals. These findings highlight a direct correlation between sarcomere length and tenderness, evident by the significant negative correlation coefficient of $r = -0.662$ ($p < 0.05$), where tender meats exhibited longer sarcomeres. This underscores the importance of sarcomere length as a critical predictor of beef tenderness.

IV. CONCLUSION

The variability in the tenderness of Brazilian beef is strongly correlated with sarcomere length. These findings offer valuable insights for the meat industry, emphasizing the significance of incorporating sarcomere length into the animal production and selection process. This approach aims to enhance meat quality and guarantee consumer satisfaction.

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ENHANCING BEEF QUALITY THROUGH EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELDS: PRELIMINARY RESULTS

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I. INTRODUCTION

The application of extremely low frequency (ELF) electromagnetic fields in meat products is novel in food science. Initial studies have shown improvements in tenderness and cooking loss of fish, particularly carp and rainbow trout [1,2]. However, the application of this technology to beef remains unexplored. Within this context, the present study aims to investigate the effects of ELF on meat quality traits of *Bos indicus* cattle.

II. MATERIALS AND METHODS

Samples of the *Longissimus thoracis* (LT) from 140 Nellore bulls, belonging to contemporary groups and feedlot finished, were used. Seventy LT were allocated for laboratory analyses (study 1), while the remaining 70 were designated for sensory analyses (study 2) of meat quality. Each portion of LT was divided into two steaks of 2.54 cm, vacuum-packed, and frozen (48h *post-mortem*). In both studies, one steak was assigned to the control treatment (CTRL), and the other to ELF. The application of ELF protocol (0 to 100 Hz) was performed using microchips, EFFATHA technology (https://www.effatha.com.br), affixed to the sample packaging. The protocol was applied after sample thawing for 14 hours. The ELF technology targets the amino acid sequences (https://www.uniprot.org/) of the proteins Actin, Myosin, Desmin, Troponin T, Titin, and Nebulin. Subsequently, all samples were aged for seven days (1 to 2° C) and used in the meat quality assays. The steaks from ELF and CTRL treatments remained in separate fridge during these processes.

In study 1, the meat pH and water-holding capacity (WHC) were measured according to procedures described in the literature [3]. Subsequently, the samples were cooked until reaching 71°C to determine the shear force (WBSF) in kilograms, following literature procedures [4,5]. Cooking losses (CL) were calculated as the difference in weight before and after cooking. In study 2, the samples were grilled until reaching 63° C and served to untrained consumers (n = 120), following literature procedures [6]. Grilled steaks were placed in small boxes (https://www.brazilbeefquality.com/) to preserve moisture and kept in ovens at 45°C to standardize the temperature. Each participant received 7 samples: one common to all (*dummy*) and six randomized samples (3 CTRL vs. 3 ELF). Consumers evaluated tenderness (TE), liking of flavor (FL), juiciness (JU), and overall acceptance (OA) on a hedonic scale from 0 to 100 points.

Analysis of variance (ANOVA) was used to test the effect of treatments on meat quality variables. Marginal means were compared using the *emmeans* function in R software (v.4.1.2). In study 2, A mixed generalized linear model was applied considering a binomial distribution using the PROC GLIMMIX procedure (SAS v.9.4). The model included sequence (sample tasting) and treatment (CTRL and ELF) as fixed effects, while consumer as random effects. For all data, significance was detected at the 0.05 level.

III. RESULTS AND DISUSSION

Meat pH was lower ($P < 0.05$) in the ELF treatment compared to CTRL (Figure 1A), which may have occurred due to the exposure of amino acids in the proteins, releasing hydrogen ions and acidifying the cellular environment, as observed in another study with pork [7]. Additionally, there was a 2% increase in WHC ($P < 0.05$) in the steaks from the ELF treatment. These results suggest modifications in the structure of the muscle tissue, affecting protein-water interaction and exposing more hydrophilic groups, which was also observed with pork [8]. Furthermore, objective tenderness improved, with a great reduction of 1.31 kg in WBSF ($P < 0.05$) in the ELF treatment versus CTRL.

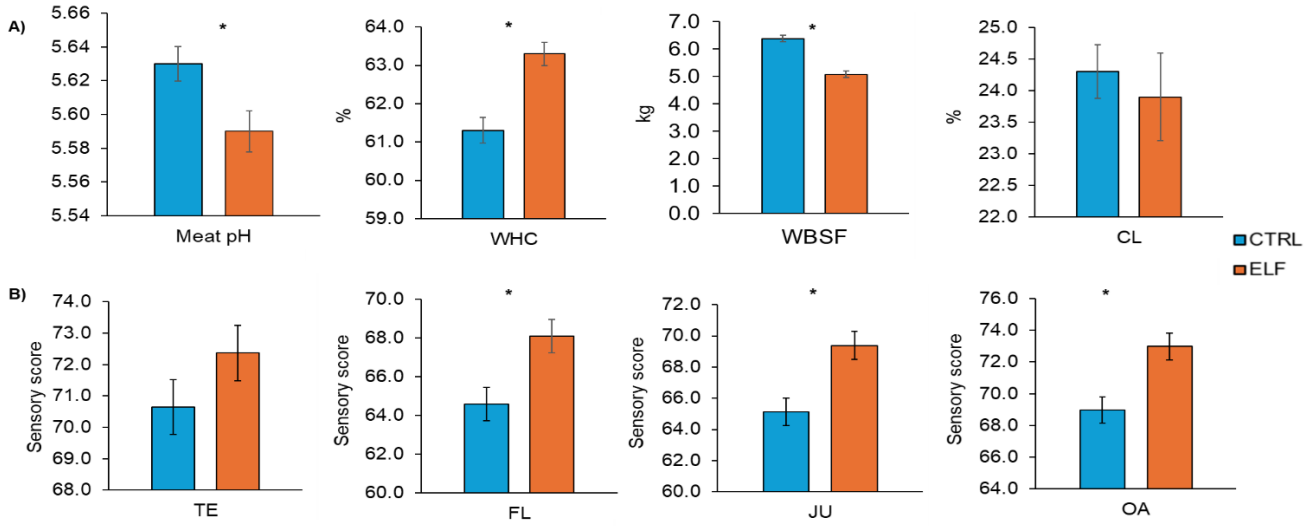

Figure 1. Results of meat quality assays (*Longissimus thoracis* [LT] samples) for control (**CTRL**) versus extremely low-frequency (**ELF** [0 to 100 Hz]) treatment. **A**) Laboratory study (n = 70 LT): WHC = Water-holding capacity; WBSF = Warner-Bratzler shear force; CL = cooking losses. **B**) Sensory study (n = 70 LT) – 120 untrained consumers (sensory score 0 to 100 points): TE = tenderness; FL = liking of flavor; JU = juiciness; OA = overall acceptance. *Significance (P < 0.05).

Positive effects (P < 0.05) on sensory traits (JU, FL, and OA) were observed (Figure 1B). Consumers gave higher ratings to steaks from ELF treatment compared to CTRL, except for TE variable. Despite its strong correlation with OA, consumers were unable to detect differences in TE alone (individually). Further research is needed to elucidate the underlying biochemical and molecular mechanisms responsible for these improvements and to optimize the application of ELF in beef [9].

IV. CONCLUSION

The use of ELF on Nellore beef reduced pH, increased WHC, and decrease WBSF, improving objective tenderness. Positive effects were also observed after ELF application on sensory traits such as juiciness, liking of flavor, and overall acceptance. These results possibly occurred due to effects on structural proteins of the LT muscle, which can be used to development of innovative strategies for enhancing meat quality of *Bos indicus* animals and meeting consumer preferences in the future.

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URUGUAYAN BEEF QUALITY AUDIT-2022: A SURVEY OF CARCASS TRAITS RELATED TO QUALITY AND VALUE OF CATTLE

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I. INTRODUCTION

The first Uruguayan Beef Quality Audit (UBQA) was conducted in 2002, in a joint project among Colorado State University, INAC e INIA and was an important benchmark to identify what the beef industry was producing, measuring and reporting on cattle and carcass traits. Seven National Beef Quality Audits (NBQA) were conducted in the U.S. (1991, 1995, 2000, 2005, 2011, 2016 and 2022), and four in Uruguay (UBQA 2002, 2007, 2013 and 2022). Many of the UBQA findings were used as training practices for producer and packers related to animal handling. But also some marketing demands have occurred, not only in Animal Welfare but also in meat quality attributes (marbling) explained by improvements in genetics and efficiency of the production systems. This UBQA-2022 was done to quantify if these mentioned changes could improve the quality and consistency of the Uruguayan cattle and identify current issues for the beef industry.

II. MATERIALS AND METHODS

Seven packing plants were visited one day in two seasons, Spring (October to December 2022) and Fall (April to June 2023). It was sampled 33% percent of the cattle from each production lot (n=3207). *Harvest Floor Assessments. Before Hide Removal*. Breed-type and crosses were classified based on visual characteristics (hide color, breed traits). Horns were evaluated visually for approximate length (none, ≤10 cm, and >10 cm). *After Hide Removal*. Liver was evaluated recording the reasons for condemnation. Carcass bruises were assessed based on location (round, loin, rib, chuck, flank, brisket, and neck), and severity (1: no tissue removal or 2: tissue removal affecting final product). *Carcass Assessments*. Beef carcasses were evaluated by Uruguayan grading system (dentition, sex, hot carcass weight HCW) [1] and by USDA Quality Grade (QG) factors (skeletal maturity and marbling) [2]. Most of these measurements were done by INAC and INIA trained personnel. *Statistical Analysis*. All analyses were performed using SAS (SAS Inst., Inc., Cary, NC). Means were generated using Proc Means and frequency distributions using Proc Freq.

III. RESULTS AND DISCUSSION

The major breed was Angus (34%) followed by Hereford (32.2%) and their crosses (26.4%), representing British breeds 92.6% of the slaughtered cattle (data not shown). Angus increased to 34% versus 17.8 % in UBQA 2013.This assessment is different to US NBQA, where hide color provides an indication of predominant breed and because it is used in branded beef programs that emphasize Angus genetics and/or black-hided cattle [3]. Black-hided cattle increased 4 % in the NBQA 2022 related to 2016 [4]. Cattle with horns can potentially cause injury or muscle bruising to other animals. UBQA showed that 82% of the cattle had not horn (71% in 2013), and 14.7% had horns >10 cm in length (20.1% in 2013). Schwartz et al. [4] reported that most cattle evaluated in NBQA 2022 had not horns (84.1%).

Inspectors from Veterinary Services determined that 25.8% of the livers were condemned. This result showed improvements in the incidence of flukes in our production systems from 2013. Schwartz et al. [4] reported an incidence rate of 28.5% for liver condemnation in NBQA 2022.

It was found that 76.4% of carcasses were bruised, being similar to UBQA 2013 (73%) . From the total carcass evaluation, 23% of them were in the round and 22.1% were in the flank. According to the severity of the bruises 74% of carcasses presented severity 1 and 26% severity 2 (within this 76.4%). In NBQA 2022, bruises were found in the loin (30%), rib (23.7%), chuck (19.7%), round (19.3%), and brisket/plate/flank (7.3%) [4].

The Official Grading System [1] classifies the carcasses by sex-classes and dentition by the number of teeth, among others. Related to sex-classes, 58.1% was steer, 11.2% was heifer and 30.7% was cow. Considering only steers, dentition distribution in UBQA 2022 were zero (6.6%), two (41%), four (22.4%), six (15.5%) and eight teeth (14.5%). It was observed a 26% decrease in the proportion of 8 teeth steers from 2013, increasing the incidence rate of 2 and 4 teeth (28%). The average HCW was 264,2 kg, being 290 kg for steers, 247 kg for heifers, and 234 kg for cows. These values are 14 kg and 10 kg heavier than 2013, for steers and cows, respectively. Lovell et al. [5] in reported an average HCW of 400.6 kg in NBQA 2022.

Frequencies of marbling scores, carcass maturity and USDA QG are shown in Table 1. An improvement was observed in score of marbling from 2013, where carcass reaching Small o higher levels increased 20%. Over 81% of the steer carcasses were A in skeletal maturity and 19% of them were B. Applying USDA QG system, most of the Uruguayan cattle was in Choice (42%), followed by Standard (22%) and Select (18.5%) grades. This data showed an increase of 24% in Choice and a decrease of 13% in Standard grade comparing with UBQA 2013. Distributions of USDA QG in NBQA 2022 were Prime (8.2%), Choice (74.7%),Select (15.8%), and other (1.4%) [5].

Table 1 - Frequencies (%) of marbling scores, skeletal maturity and USDA Quality Grade for all carcasses. UBQA 2022.

Marbling	Freq.	Skel. Mat.	Freq.	USDA QG	Freq.
Pd	1.3	A	61.4	Prime	1.2
Tr	10.9	B	21.9	Choice	42.3
Sl	30.8	C	10.9	Select	18.5
Sm	30.6	D	5.6	Standard	22.0
Mt	17.7	E	0.2	Commercial	4.0
Md	6.8			Utility	11.5
SlA/MdA	1.9			Cutter	0.5

IV. CONCLUSION

Uruguayan Beef Quality Audits are a mean to identify the main problems for the beef industry and how they affect the value of live cattle, carcasses, or by-products. Carcass bruising resulted in economic loss to the beef industry and an animal-welfare concern. UBQA 2022 produced higher average hot carcass weight and marbling scores, increasing the contribution of young animals related to 2013. Also, it was observed an increase in the percentage of USDA Choice carcasses from 2013. This information will help the Uruguayan beef industry to evaluate the beef quality progress and provide a benchmark for future educational and research programs.

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A SECOND LOOK INTO THE UNDERLYING BIOCHEMICAL MECHANISMS OF ELECTRICALLY STIMULATED CARCASSES AS REVEALED BY PROTEOMICS: RESULTS OF AN INTEGRATIVE ANALYSIS ON CATTLE

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I. INTRODUCTION

Meat eating quality traits such as tenderness, flavor, and juiciness significantly influence beef palatability [1]. Research on meat consumption trends has revealed that beef tenderness is crucial for consumers' satisfaction, and they are willing to pay more if tender is guaranteed [1]. However, beef tenderness is variable due to multifaceted factors [2]. Several post-mortem interventions can be used to enhance beef tenderization, among which electrical stimulation (ES) has revolutionized meat processing [3]. Initially proposed to prevent cold shortening, ES accelerates proteolysis and myofibril degradation, thereby improving tenderness. However, the mechanisms behind ES were mostly described using traditional biochemistry methods, and very recently using proteomics. This paper aims to gather and reuse the published ES bovine proteomics studies to provide novel insights and a second look into the molecular pathways using integrative proteomics and in-depth bioinformatics.

II. MATERIALS AND METHODS

Through an integromics approach [4] on published ES proteomics studies, 6 papers that all applied low-voltage ES (LVES) to investigate the proteome changes triggered by stimulation in post-mortem bovine muscle have been gathered. The inclusion/exclusion criteria were based on i) proteomics on *Longissimus* muscle, ii) only proteins that were changing in response to LVES, and iii) exclusion of papers not in the frame of MeatOmics. Subsequently, we created the first compendium of *Longissimus* bovine muscle proteins that change in abundance due to LVES. The compendium was subjected to in- depth bioinformatics for i) manual annotation into pathways of the proteins using the gene ontology (GO) of UniProt KB (https://www.uniprot.org/), ii) protein-protein interactions using STRING database (https://string-db.org/), and GO enrichment analysis using Metascape® (https://metascape.org/).

III. RESULTS AND DISCUSSION

The compendium gathered 67 interconnected proteins belonging to 7 biological pathways (Figure 1A), from which 14 were consistently identified across the 6 studies. Shared molecular pathways features emerged (Figure 1B). First, in line with the purpose of ES, the most enriched GO term was "ATP metabolic process" (Figure 1C), common to 4 studies (Figure 1B). Interestingly, half of the common proteins were glycolytic enzymes and all were from the payoff phase of glycolysis (Figure 1D). Creatine kinase M-type (CKM) is the 7th and top protein that did not belong to glycolysis, but was found down-regulated in all ES studies (Figure 1D). Second, the "muscle system process" constituted the 2nd major pathway with 3 consistently identified proteins (ACTA1, MYL2 and MYLPF). The enhanced tenderization ascribed to ES was concomitant with the expression level of small heat shock proteins (CRYAB, HSPB1 and HSPB6, Figure 1D) and their enrichment across multiple studies (Figure 1E). The early post-mortem involvement of both energy metabolic pathways and cytoskeletal proteins as a result of ES may support apoptosis onset. In fact, two GO terms related to apoptosis regulation were significantly enriched (Figure 1B). Further, the results evidenced a sophisticated interplay among proteolysis, muscle structure, and responses to cellular and oxidative stress in ES muscle. As a proof- of-concept within the realm of data reuse, we demonstrated the first interconnectedness in the molecular signatures triggered by LVES. It further elucidated the intricate biochemical mechanisms underlying ES, likely amplifying apoptosis onset and its consequences on beef tenderization.

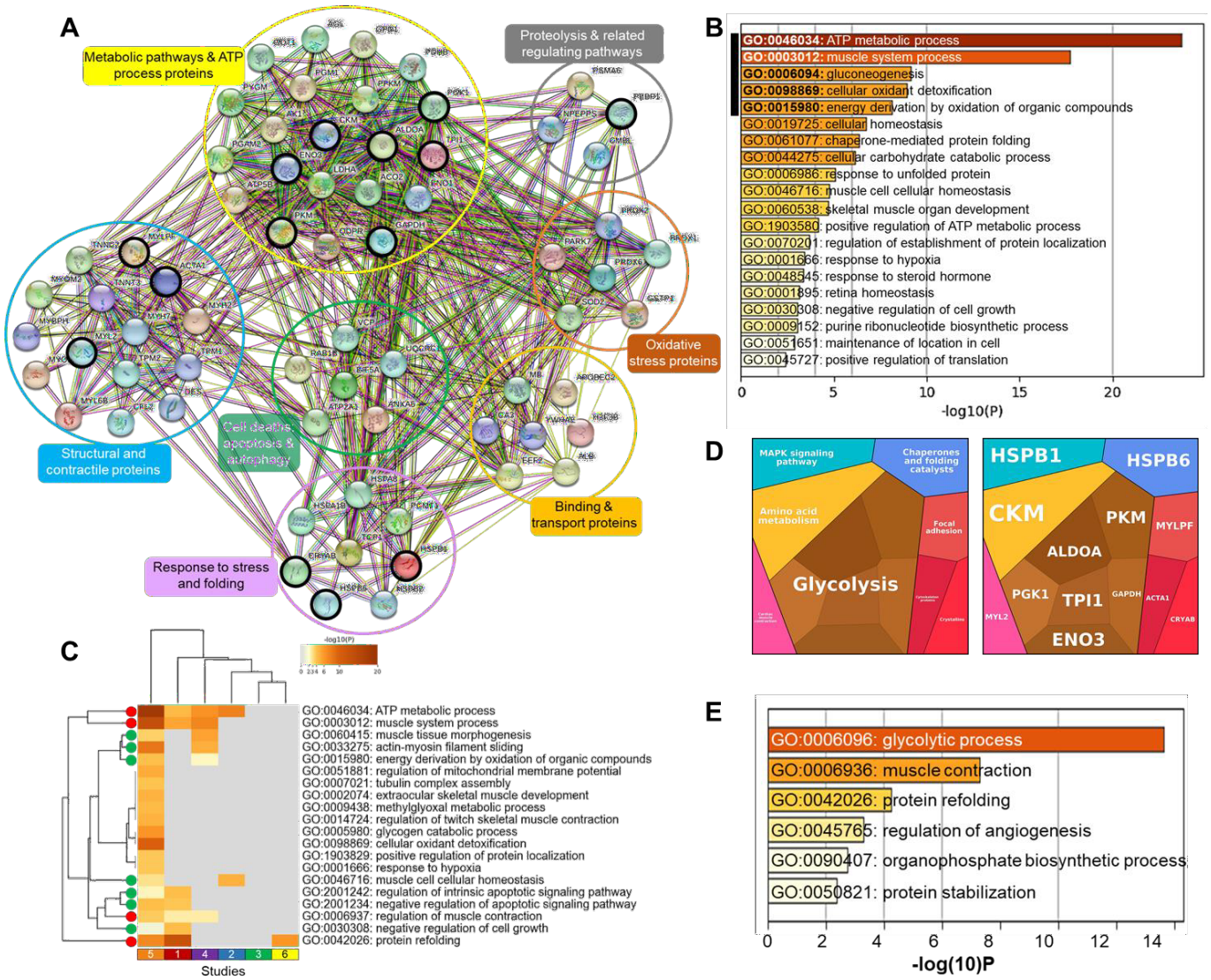

Figure 1. **A)** String protein-protein interaction (PPI) network (n=67 proteins). Proteins are clustered into 7 annotated biological pathways. Proteins (n=14) identified ≥2 times are indicated with black circles. **B)** GO enrichment analysis using Metascape®. **C)** Heatmap clustering comparing the enriched GO terms across studies. GO terms common to ≥ 3 studies are in red solid circles and those common to 2 studies are in green. **D)** Proteomaps analysis highlighting the enriched KEGG pathways and proteins consistently identified. **E)** GO enrichment based on the common 14 proteins using Metascape®.

IV. CONCLUSION

This study is the first to reveal, using in-depth bioinformatics, the molecular pathways and signatures behind LVES applied to bovine carcasses. The findings contribute to a better understanding of the complex biochemical processes involved in post-mortem muscle metabolism and their impact on meat tenderization, promoting evidence-informed strategies for optimizing meat processing techniques. The application of metabolomics and shotgun proteomics would allow decipher further mechanisms, including differences, behind ES systems and extend our understanding of the factors at interplay.

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NADPH IMPROVES RED COLOR FORMATION IN BEEF INCUBATED WITH NO-SYNTHASE AND L-ARGININE.

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I. INTRODUCTION

Numerous methods have been proposed to reduce nitrite levels in meat products or find nitrite alternatives (1). Our proposition involves utilizing the enzyme Nitric oxide (NO) synthase, naturally present in living cells, and crucial in various physiological processes including blood pressure regulation, immune response, and neuronal signaling. NO synthase catalyzes the conversion of arginine into nitric oxide under specific conditions, dependent on the isoform of the enzyme: inducible (iNOS), endothelial, or neuronal (2). This study extends our previous research and aims to evaluate the effects of varying time, enzyme, and arginine concentrations. NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate serves as a cofactor for iNOS. Given its ubiquitous presence in mammalian cells, (in the initial phase of the experiment) we anticipated its existence in the meat used for our experiment. In this phase of of our research, we investigated changes in meat color in the presence or absence of NADPH.

II. MATERIALS AND METHODS

Beef *m. semimembranosus* from a local retailer (Makro, Poland) was ground (3 mm plate) (Mado, Germany). The whole mass was mixed, divided into 50 g portions, and placed in glass beakers along with enzyme (iNOS- 1U = 254mg of protein), L-arginine and/or NADPH (Table 1).

Table 1. Additives used in the test samples with NADPH (per 50g portion)

Sample ID	iNOS [U]	L-Arginine [g]	NADPH [g]	Time [h]	Sample ID	iNOS [U]	L-Arginine [g]	NADPH [g]	Time [h]
1	0.5	0.05	0.06	3h	12	0.5	0.05	0.06	6h
2	0.5	0.25	0.06		13	0.5	0.25	0.06	
3	0.5	0.75	0.06		14	0.5	0.75	0.06	
4	1.0	0.05	0.12		15	1.0	0.05	0.12	
5	1.0	0.25	0.12		16	1.0	0.25	0.12	
6	1.0	0.75	0.12		17	1.0	0.75	0.12	
7	1.5	0.05	0.18		18	1.5	0.05	0.18	
8	1.5	0.25	0.18		19	1.5	0.25	0.18	
9	1.5	0.75	0.18		20	1.5	0.75	0.18	
10	No additives				21	No additives			
11	4 mL $NaNO_2$ (1g/L)				22	4 mL $NaNO_2$ (1g/L)			

Meat was incubated at 37 °C for 3 h or 6 h and cooked afterwards (95°C/30 minutes), cooled down and stored (4°C). Color of the samples was measured (Konica Minolta CM-3500d colorimeter, Osaka, Japan) (measurement mode: D/8 (SCE), D65 illuminate,10° viewing angle. L*, a*, and b* coordinates were measured. Redness (a*/b*) and Chroma ($C= [a^{*2}+v^{*2}]^{0.5}$) were calculated. The results were subjected to ANOVA analysis, the differences were tested using Tukey test at $P<0.05$.

III. RESULTS AND DISCUSSION

All samples containing 1.5% L-Arginine showed notably higher a* values (Table 2) and redness (a/b) values compared to samples with lower L-Arginine concentrations. This supports the findings of Bludau et al.,(3) who investigated beef frankfurters with L-Arginine addition and achieved satisfactory results, albeit lower than nitrite-cured sausages. Addition of NADPH to all samples resulted in increased redness, although statistically significant differences were only observed for samples incubated for 3 hours with 1U of NO synthase (samples 4, 5, and 6), or with 1.5 U of NO synthase

and 1.5% L-Arginine, as well as for samples incubated for 6 hours with either 1 or 1.5U of NO synthase and 1.5% L-Arginine. These findings suggest that NADPH may enhance NO synthase activity, particularly at higher enzyme and L-Arginine concentrations, when sufficient iNOS and its substrate are available. Samples 6, 20, 17, 3, and 9 exhibited the highest redness values, although these values were only around 60% of those obtained for nitrite-cured samples.

Table 2. CIELab parameters of meat samples incubated with or without NADPH (mean values ± standard errors).

Sample ID	L*(D65)		a*(D65)		b*(D65)		a*/b*	
	no NADPH	NADPH	no NADPH	NADPH	no NADPH	NADPH	no NADPH	NADPH
1	46.1 ±0.7	45.3 ±1.0	6.4 ±0.1	6.8 ±0.1	15.3 ±0.2	14.6 ±0.2	0.4 ±0.0	0.5 ±0.0
2	46.3 ±0.8	44.8 ±0.9	6.9 ±0.1	7.3 ±0.1	15.0 ±0.2	14.1 ±0.3	0.5 ±0.0	0.5 ±0.0
3	42.4 ±0.6	43.3 ±0.7	7.6 ±0.1	8.6 ±0.1	13.0 ±0.3	13.7 ±0.2	0.6 ±0.0	0.6 ±0.0
4	48.0 ±0.5	44.7 ±0.9	6.1 ±0.1	6.9 ±0.2	14.7 ±0.2	14.0 ±0.3	0.4 ±0.0	0.5 ±0.0
5	45.8 ±0.9	45.3 ±0.8	7.0 ±0.1	7.3 ±0.1	15.1 ±0.2	14.1 ±0.3	0.5 ±0.0	0.5 ±0.0
6	42.6 ±0.7	41.5 ±0.6	7.6 ±0.1	8.3 ±0.2	13.2 ±0.2	12.0 ±0.2	0.6 ±0.0	0.7 ±0.0
7	45.9 ±1.2	47.0 ±0.4	6.3 ±0.2	6.5 ±0.1	14.8 ±0.3	14.3 ±0.1	0.4 ±0.0	0.5 ±0.0
8	46.5 ±0.6	45.6 ±0.4	6.4 ±0.2	7.0 ±0.1	14.5 ±0.2	14.7 ±0.2	0.4 ±0.0	0.5 ±0.0
9	44.0 ±0.4	43.4 ±0.4	7.2 ±0.1	8.2 ±0.1	13.6 ±0.3	13.3 ±0.4	0.5 ±0.0	0.6 ±0.0
10	46.3 ±0.9	43.6 ±0.9	6.1 ±0.1	6.4 ±0.1	14.6 ±0.3	14.1 ±0.4	0.4 ±0.0	0.5 ±0.0
11	43.1 ±0.7	45.6 ±0.6	13.6 ±0.3	13.9 ±0.2	12.6 ±0.1	12.4 ±0.1	1.1 ±0.0	1.1 ±0.0
12	**47.8 ±0.8**	**50.2 ±0.6**	**6.3 ±0.1**	**6.1 ±0.1**	**15.2 ±0.2**	**14.6 ±0.2**	**0.4 ±0.0**	**0.4 ±0.0**
13	45.8 ±0.9	47.8 ±0.7	6.6 ±0.2	6.7 ±0.1	15.2 ±0.2	14.0 ±0.2	0.4 ±0.0	0.5 ±0.0
14	42.4 ±0.6	43.8 ±0.3	7.6 ±0.1	7.8 ±0.1	13.1 ±0.3	13.3 ±0.2	0.6 ±0.0	0.6 ±0.0
15	46.6 ±1.2	48.6 ±1.0	6.8 ±0.3	5.8 ±0.1	15.7 ±0.3	13.8 ±0.3	0.4 ±0.0	0.4 ±0.0
16	46.8 ±0.6	48.9 ±0.4	6.7 ±0.1	6.4 ±0.1	15.1 ±0.2	13.7 ±0.3	0.4 ±0.0	0.5 ±0.0
17	43.1 ±0.7	43.4 ±0.6	7.2 ±0.1	7.6 ±0.2	13.0 ±0.4	12.1 ±0.3	0.6 ±0.0	0.6 ±0.0
18	47.9 ±0.5	49.7 ±0.5	6.0 ±0.1	6.0 ±0.1	15.3 ±0.1	14.1 ±0.2	0.4 ±0.0	0.4 ±0.0
19	46.4 ±0.5	48.6 ±0.5	6.7 ±0.2	6.6 ±0.1	15.5 ±0.1	14.4 ±0.2	0.4 ±0.0	0.5 ±0.0
20	42.2 ±0.5	44.1 ±0.8	7.4 ±0.1	7.8 ±0.1	13.3 ±0.2	12.1 ±0.3	0.6 ±0.0	0.7 ±0.0
21	48.9 ±0.1	48.2 ±0.8	5.7 ±0.1	5.6 ±0.1	15.1 ±0.1	13.9 ±0.3	0.4 ±0.0	0.4 ±0.0
22	**45.6 ±0.5**	**47.2 ±0.7**	**13.7 ±0.2**	**12.8 ±0.3**	**12.6 ±0.01**	**11.4 ±0.2**	**1.1 ±0.0**	**1.1 ±0.0**

pH of the samples ranged from 5.9 (11,12, 21, 22) to 6.8-7.0 (3, 6, 9, 14, 17, 20). pH increase is usually associated with higher redness (4).Since these samples also had higher pH compared to the others, it raises the question if the improved redness can be solely attributed to the higher pH.

IV. CONCLUSION

NADPH enhances the color of meat when higher levels of iNOS (1.0 and 1.5U) and L-Arginine (1.5g/100g of meat) are present. The a/b values saw an increase of 12.3-19.8% compared to samples without NADPH. However, these values were only about 60% of those in cured samples.

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THE QUALITY OF STEAKS PRODUCED FROM DAIRY VS BEEF CATTLE BREEDS

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I. INTRODUCTION

Beef has been an important element of the diet and is characterized by undeniable nutritional value and unique organoleptic properties, being a source of high-biological-value protein, polyunsaturated fatty acids, fat-soluble vitamins and many bioactive nutrients and antioxidants [1,2]. Beef production in Poland is based on dairy or dual-purpose cattle herds. As much as 93% of the total amount of cattle in the country is dairy cattle, making the Polish Holstein-Friesian (PHF) the main source of beef. Among beef breeds, Limousine (LM), Hereford (HH), and Charolais (CH) are the most popular in Poland [2]. Since beef from dairy breeds is of unmatched quality, technological solutions are being sought to improve its culinary quality. One method is sous-vide cooking, which is recommended to improve the quality of beef [3]. Therefore, it was hypothesized that beef steaks, both from beef breeds (LM) and dairy breeds (PHF), subjected to sous-vide processing would have similar tenderness and juiciness.

II. MATERIALS AND METHODS

The steaks were prepared from 31 *longissimus lumborum* (LL) muscles from young (approximately 21 months old) Limousine bulls (LM, beef breed, n=14) and Polish Holstein-Friesian bulls (PHF, dairy breed, n=17). The experimental material included bulls, equally reared and fed at the Agricultural Experiment Station in Bałcyny (Poland). Details of breeding, slaughter and sampling procedures are described in the previous article [4]. Wet ageing was carried out at 4°C for 14 days. The samples were then frozen (-18°C) until the experiment was carried out. To characterize the raw material, its chemical composition, pH and color were examined [4]. In order to verify the hypothesis, 2.5 cm steaks were subjected to 2 types of thermal treatment: in individual plastic bags, submerged in a water bath (WB) at a temperature of 80°C, and kept for 40 min and vacuum-packaged products cooked in the sous-vide (SV) device at 60 °C for 4 h [4]. Warner-Bratzler Shear Force (WBSF) was determined using Instron 5942 (Instron, Norwood, USA), whereas tenderness, juiciness and intensity of meat flavor were assessed sensory on a 10-point scale sensory [4]. Statistica 13.3 was used for the data analysis. The data were subjected to analysis of variance using ANOVA, by considering a level of significance $p < 0.05$. To compare sensory analysis results, non-parametric Mann-Whitney U test was applied.

RESULTS AND DISCUSSION

Meat raw material from the LM breed was characterized by a significantly higher water content of 74% (LM) vs 72% (PHF) and protein content of 23.7% vs 22.8% respectively, a lower fat content of 2% (LM) vs 4.4% (PHF), and a similar level of ash content – 1%, which is comparable to the results of other researchers [2]. The LL muscle from both breeds was characterized by the same lightness L*, while the raw material from the PHF breed was more red (a*) and yellow (b*) compared to the LM breed. Muscle pH was 5.58 LM and 5.45 PHF. The analysis of the results after the applied thermal treatments allowed for the conclusion, that the breed influenced all (except tenderness after heating in WB) tested parameters of the steaks shown in Table 1. Regardless of the type of thermal treatment,

LM steaks recorded higher scores for the examined characteristics in the sensory assessment, as well as lower WBSF by 44% (WB) and 19% (SV), which indicates better tenderness of LM steaks. The type of thermal treatment also significantly influenced the tested parameters (except meat taste intensity-PHF), giving better results for steaks cooked using the sous-vide method - Table1. Despite the use of sous-vide cooking, the panelists gave significantly higher scores to steaks prepared from the LM meat breed compared to those from the dairy breed - tenderness 7.7 (LM) vs 5.96 (PHF) and juiciness 7.28 vs 5.28, respectively.

Table 1 – Effect of cattle breed and thermal treatment on Warner-Bratzler shear force (WBSF) and sensory-assessed juiciness, tenderness, and meat taste intensity (mean values and standard error of the mean SEM).

Attribute	Thermal treatment method (T)	Cattle breed (B)				P value
		PHF (dairy)		LM (beef)		
		$\bar{x}$	SEM	$\bar{x}$	SEM	B
WBSF, N	WB	55.5	1.4	36.6	0.8	*
	SV	31.5	1.6	25.6	0.8	*
	P value T	*		*		
Sensory quality						
Juiciness, points	WB	2.67	0.13	4.67	0.16	*
	SV	5.28	0.15	7.28	0.15	*
	P value T	*		*		
Tenderness, points	WB	5.01	0.16	4.77	0.18	NS
	SV	5.96	0.19	7.70	0.19	*
	P value T	*		*		
Meat taste intensity, points	WB	6.37	0.15	8.02	0.12	*
	SV	6.50	0.11	8.69	0.11	*
	P value T	NS		*		

* Difference significant at $p < 0.05$. NS = no significant difference. HO- Holstein-Friesian bulls; LM – Limousine bulls; WB - water bath; SV – sous-vide; Sensory assessment on 1–10 scale, juiciness (1, extremely dry; 10, extremely juicy), tenderness (1, extremely tough; 10, extremely tender), meat taste intensity (1, imperceptible; 10, extremely intense)

III. CONCLUSION

The results of the present study do not confirm the formulated hypothesis. Despite the use of the sous-vide method, steaks prepared from Limousine beef were characterized by much better tenderness, juiciness and intensity of meat flavor in sensory evaluation as well as lower shear force in instrumental evaluation compared to steaks from Polish Holstein-Friesian dairy breed. Therefore, there is a need for further research and to propose other technological treatments that could equalize the quality of dairy and meat breed steaks.

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Effect of intermediate ultimate pH beef over aging time on *Longissimus lumborum* muscle proteome from grass-fed Nellore

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I. INTRODUCTION

The ultimate pH (pHu) plays an important role in beef quality. Several factors such as animal diet, exercise and pre-slaughter stress can result in muscle glycogen depletion and cause an abnormal pHu beef (> 5.80) [1]. In Brazil, the incidence of beef classified as intermediate (5.8 to 6.19) or high pHu (≥ 6.2) represents approximately 40% of production, resulting in large economic losses [2]. Therefore, the aim of this work was to evaluate the effect of intermediate pHu beef over aging time on *Longissimus lumborum* (LL) muscle proteome from grass-fed Nellore (*Bos indicus*) bulls.

II. MATERIALS AND METHODS

Three LL muscles from grass-fed Nellore bulls (30 - 35 months old) classified as intermediate pHu (pHu 5.8 to 6.19) were obtained from a commercial slaughterhouse. The muscles were divided into 2.5 cm thick steaks and assigned to a treatment: 1-d (72 h *post-mortem*) and 14-d of vacuum aging at 4°C. Samples from each aging time were stored at −80 °C for proteomic analysis. Protein extraction was performed as described by Wiśniewski *et al*. [3]. LC-MS/MS analysis was performed on a Xevo G2-QTof mass spectrometer (Waters Coorporation) coupled directly to the chromatographic system. Differentially abundant proteins (DAPs) over aging time were defined through volcano plot analysis (fold change ≥ 1.2; $P \leq 0.05$). Enriched Gene Ontology terms and pathways were investigated using the open-source tool Metascape® ($P \leq 0.05$, minimum overlap of 3, and enrichment factor > 1.5).

III. RESULTS AND DISCUSSION

PCA discriminated beef at 1-d and 14-d aging (Fig. 1A). Volcano plot analysis (Figure 1B) revealed 26 DAPs between aging times comparison, of which 12 were over abundant at 1-d aging and 14 were over abundant at 14-d aging. Enrichment analysis revealed 10 enriched terms (Figure 1C), in which most of them were related to energy metabolism and muscle structure. Succinate-CoA ligase subunit alpha (SUCLG1), NADH dehydrogenase ubiquinone flavoprotein 2 (NDUFV2), ubiquinone biosynthesis monooxygenase COQ6 (COQ6) and NADH dehydrogenase ubiquinone 1 subunit C2 (NDUFC2) were abundant at 1-d aging indicating increased oxidative metabolism, as also observed by Zhai et al. [4]. Suggesting increased oxidative stress in meat with intermediate pH resulting in reduced proteolysis and degradation of structural proteins. The overabundance of candidate biomarkers for tenderness such as aldehyde dehydrogenase (ALDH2), myozenin-1 (MYOZ1), malate dehydrogenase, mitochondrial (MDH2), troponin T (TNNT1) and underabundance of heat shock protein HSPA5 observed in intermediate pHu beef at 14-d aging compared to 1-d aging are indicative of a delay in tenderization, which partially explain the toughness of intermediate pHu beef, as reported by [1, 5].

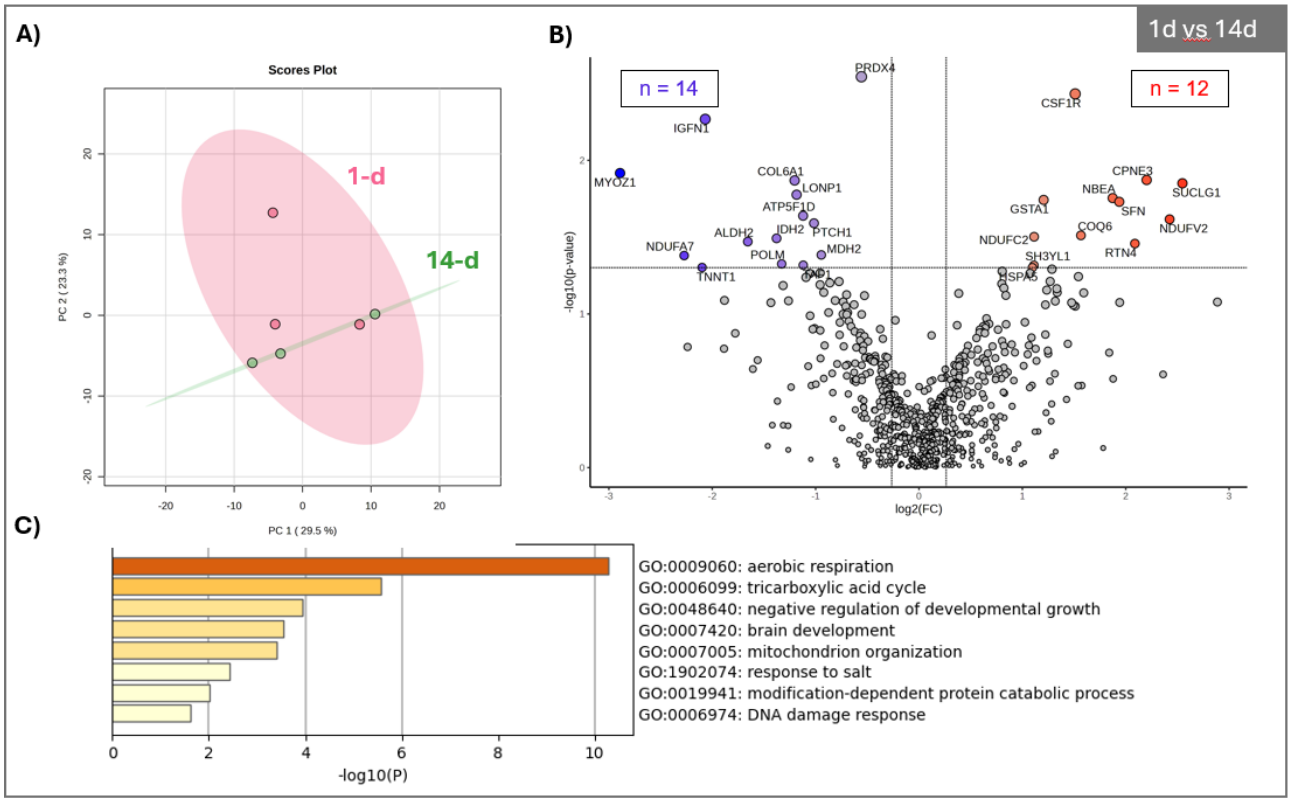

Figure 1. A) Principal Component Analysis (PCA); B) Volcano plot showing DAPs between 1 and 14 days of aging; C) Bar chart of significantly enriched GO cluster terms according to *P*-values ($P \leq 0.05$).

IV. CONCLUSION

The main proteomic changes of intermediate pHu beef over aging time are related to energy metabolism and muscle structure, revealing some proteins important for the main meat quality attributes, such as beef color and tenderness.

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EFFECT OF TIME AND TEMPERATURE ON THE PROFILE OF VOLATILE COMPOUNDS IN BEEF PROCESSED BY THE *SOUS VIDE* METHOD

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I. INTRODUCTION

Sous vide is a term of French origin that means “under vacuum”. It is a cooking technique that preserves the integrity of food by heating it for long periods at relatively low temperatures [1]. This technique is applied to extend the shelf life of food, avoiding waste and reducing the loss of liquid, nutrients and aromas sensitive to heat. Different temperatures and cooking times can modify the profile of volatile compounds and, consequently, the *flavor* of cooked meat [2]. Although there are several studies on the formation of the profile of volatile compounds in meat cooked using traditional methods, few articles report the formation of volatile compounds in beef cooked *sous vide* at low temperatures and for long periods. This work aimed to evaluate the effect of different combinations of time and temperatures on the formation of volatile compounds in beef cooked by the sous vide method.

II. MATERIALS AND METHODS

M. *Biceps femoris* pieces were purchased from a commercial slaughterhouse, divided into 2.5 cm thick steaks, and vacuum packed. Cooking was carried out using the *sous vide* technique, using a thermocirculator at different combinations of time and temperature: 60°C/60 min; 60°C/360min; 65°C/210min; 70°C/60min and 70°C/360min. After cooling the samples at 3°C in an ice bath, the samples were ground in a food processor. One g of the ground sample was weighed in a glass flask with a capacity of 60 mL, and the extraction of volatile compounds was performed by the technique of solid phase microextraction (SPME) using a carboxy/polydimethylsiloxane (CAR/PDMS) *fiber* as stationary phase. Gas chromatography coupled to mass spectrometry (GC-MS) was used to separate and identify volatile compounds in the samples, using a DB-5 MS column (5% phenyl, 95% dimethylpolysiloxane) 60 m x 0.25 mm internal diameter and one µm stationary phase thickness. The oven temperature started at 40 °C, increasing 4 °C min^{-1} to 180 °C, 10 °C min^{-1} to 280 °C, remaining at this temperature for 5.3 min. Helium (He) was used as carrier gas. The compounds were identified through their spectra and compared with those of the NIST library database. To confirm, an n-alkane (C7-C30) solution (Supelco, Bellefonte, PA) was injected into the equipment under the same conditions as the samples to obtain the programmed linear retention temperature index (LTPRI) of volatile compounds. Experimental identification was performed by comparing the LTPRI and mass spectra with literature reports, with a minimum similarity of 85%. A qualitative analysis was applied to analyze the obtained data.

III. RESULTS AND DISCUSSION

A total of 80 volatile compounds were identified in beef samples cooked by the *sous vide* method at different time and temperature combinations. The compounds were classified as alcohols (n=12), aldehydes (n=13), carboxylic acids (n=6), esters (n=17), hydrocarbons (n=14), ketones (n=11),

lactone(n=1), sulfur compounds (n=2) and terpenes (n=2). The number of compounds of each chemical class per treatment can be seen in Table 1.

Table 1 – Chemical class of volatile compounds in beef samples in *sous vide* cooked at different time and temperature combinations.

Class	**60°C/60min**	**60°C/360min**	**65°C/210min**	**70°C/60min**	**70°C/360min**
Alcohol	4	6	9	8	7
Aldehyde	6	11	8	7	7
Carboxylic acid	1	2	5	2	3
Ester	6	13	15	7	7
Hydrocarbon	7	12	3	3	5
Ketone	7	9	9	7	6
Lactone			1		
Sulfur compound		2	2	2	2
Terpene			2		
Total compounds	31	55	54	36	37

In the distribution of different volatile compound classes under various cooking conditions, it is observed that the treatment 60°C/360min and 65°C/210 min resulted in the highest total detected number of compounds, with notably higher counts of esters, aldehydes and ketones, followed by alcohols. Conversely, fewer compounds were identified in the treatment with a shorter time and temperature (60°C/60min). Some classes, such as lactones and terpenes, were only detected under specific conditions (65°C/210 min). Increasing the cooking temperature over long periods influences flavor development through the Maillard's reaction and lipid oxidation [3]. According to Roldan [4], *sous-vide* cooking at moderately high temperatures for extended periods of time stimulates the formation of volatile compounds from reactions involved in amino acids that form a desirable meaty *flavor*.

IV. CONCLUSION

The volatile compounds profile in beef cooked by the *sous vide* method was influenced by cooking time and temperature. This highlights the importance of controlling cooking parameters to achieve desired flavor profiles in beef *sous vide* cooking.

ACKNOWLEDGEMENTS

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THE USE OF ^{1}H NMR IN THE QUANTIFICATION OF FREE AMINO ACIDS IN DRY-AGED AND WET-AGED BEEF

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I. INTRODUCTION

Meat from *Bos indicus* cattle has been associated with lower tenderness than *Bos taurus* cattle [1]. Wet and dry aging processes have been used to improve sensory attributes such as flavor and tenderness. During aging, enzymatic proteolysis promotes the release of free amino acids, which influence the flavor of meats, such as sweet taste (glycine, alanine, serine, threonine, proline, and hydroxyproline), sour taste (phenylalanine, tyrosine, and alanine), bitter taste (histidine, arginine, isoleucine, leucine, lysine, phenylalanine, tyrosine, valine), and umami (glutamic acid and aspartic acid). Differences in the amount and type of free amino acids, between breeds and the aging process, may be responsible for differences in sensory attributes. This research aims to evaluate beef from crossbred *Bos taurus* x *Bos indicus* animals and Nellore breed, aged via dry-aging and wet-aging, comparing changes in the profile of free amino acids.

II. MATERIALS AND METHODS

Ten samples were obtained from crossbred castrated male cattle, with at least 50% *Bos taurus* content, kept for at least 120 days in feedlots during finishing, and at 24 months of age. Additionally, eight samples were taken from intact male Nelore cattle raised in a semi-confined system, also aged 24 months. After slaughter and chilling (2°C, 48 hours), samples were collected from the m. *Longissimus thoracis et lumborum* between the 9th thoracic vertebra and the 3rd lumbar vertebra. Samples were separated for evaluation without aging, and the rest were aged for 28 days, divided into two treatments, wet-aging and dry-aging, in an aging chamber (10°C ± 1°C / ± 75% RH). ^{1}H NMR spectra were acquired on a Bruker Avance III 500 spectrometer at 11.75 T, using the BBI probe at 25°C, after the extraction process in 200 mg of meat with water and chloroform. An aliquot of the aqueous phase was transferred to the NMR tube and homogenized with 200 µL of D2O/DSS/Sodium Azide solution. The spectra were processed using Topspin 3.1 software. Chenomx NMR Suite 8.3 software and literature data (Human Metabolome Database (HMDB)) were used to assist in compound identification. Statistical analyses (ANOVA and Tukey HSD test) were performed using Statistica 7 software.

III. RESULTS AND DISCUSSION

The aging processes increased the content of most free amino acids (Figure 1). Dry-aged samples showed a more pronounced increase in amino acid content than wet-aged samples, regardless of animal genetics. When comparing crossbred with Nellore beef, it is noticeable that all amino acids presented more expressive increases in the crossbred beef, regardless of the aging type (dry-aged and wet-aged). This more expressive increase in free amino acid content may influence the more pronounced flavor of dry-aged beef. The most pronounced changes in concentrations of free amino acids during dry aging may be due to two factors: the action of microorganisms on the crust, which can intensify proteolysis, and concentration of compounds by moisture evaporation [2]. The loadings plot (Figure 2) shows that all amino acids are grouped in the same PC of crossbred dry-aged beef (positive PC2 and negative PC1), showing a higher correlation of these compounds with this group, which reinforces the possibility of these free amino acids influencing sensory attributes. Unaged crossbred and Nellore samples (positive PC1 and PC2) were also grouped, indicating similar composition

characteristics. Nellore dry-aged and wet-aged samples were also grouped (positive PC1 and negative PC2). Finally, crossbred dry-aged and wet-aged beef are grouped only in negative PC1.

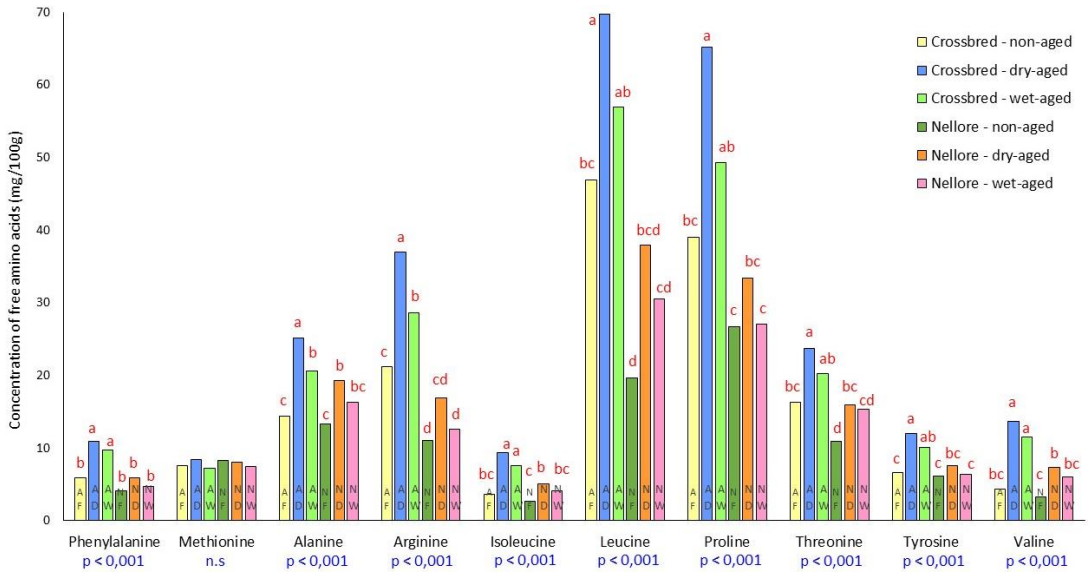

Figure 1. Concentration of free amino acids (mg/100g) in Crossbred and Nellore beef during dry- and wet-aging (28 days).

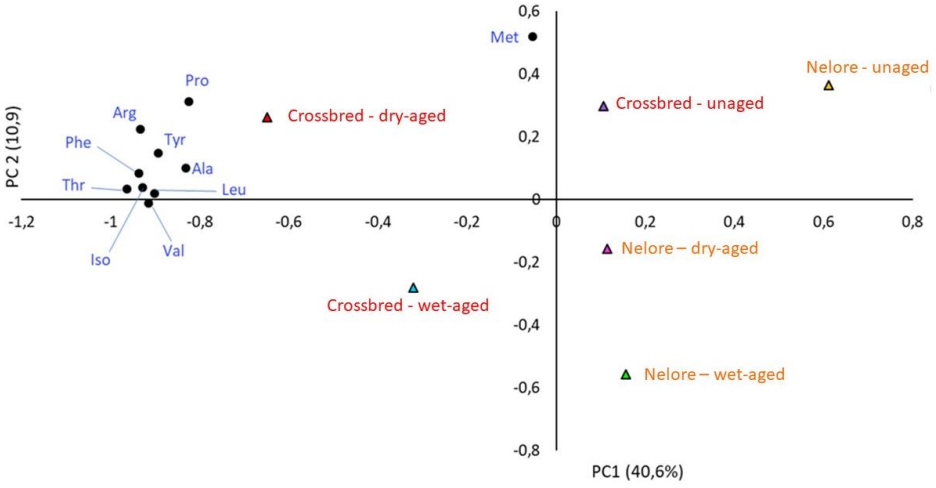

Figure 2. Plot of the variables having the highest discrimination power with significantly differences (P<0.05) among the groups, according to the principal components loading vectors.

IV. CONCLUSION

Through ^{1}H NMR analyses, it was possible to identify that beef from crossbred animals had more free amino acids than Nellore. Additionally, dry-aging resulted in a greater quantity of free amino acids compared to wet-aging.

ACKNOWLEDGEMENTS

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DETERNIMATION OF ALDOSES IN BEEF SAMPLES BY APPLYING AN ALDONONITRILE ACETATE DERIVATIZATION METHOD

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I. INTRODUCTION

The aroma of beef is known to be caused by the perception of odor-active volatiles, which are formed during cooking and then released from beef during eating. In making process of corned beef products, a sterilization of sealed can contain beef and other ingredients is conducted with high-temperature and high-pressure steam. The heating treatment might contribute to generate its characteristic roast odor from beef. Our previous study showed that pyrazines, roast meat-like aroma, were suggested to be a candidate responsible for the aroma [1]. Pyrazines would be formed by an amino-carbonyl reaction between amino acids and sugars in beef, however, the underlying mechanisms of aroma generation in beef with heating have not been clarified enough. To date, a variety of analytical techniques have been employed to determine the monosaccharide composition. In metabolomics studies, metabolites are usually derivatized with an oximation reagent followed by silylation. One drawback of this method is the silylated derivatizes exist in anomeric forms, which leads to multiple peaks on GC/MS analysis. The aldononitrile acetate derivatization (AND) has been also used for a monosaccharide composition analysis from soil or plant samples [2,3]. The derivatized aldose produces a unique single peak in a GC/MS analysis. Therefore, in this study, we tried to quantify aldose component in beef sample by using AND approach.

II. MATERIALS AND METHODS

The AND was performed as previously described [2,3] with some modifications. Briefly, the standard monosaccharides were incubated with a derivatization reagent (30 mg/mL hydroxylamine hydrochloride in pyridine) at 60°C for 30 min. Then, for acetylation of aldononitrile derivatives, acetic anhydride was subsequently added and incubated at 30°C for 10 min. After the derivatization step, dichloromethane was added to extract the acetylated derivatives. The solution was washed with deionized water for two times. Finally, 1 µL of the dichloromethane layer was subjected to GC/MS analysis. Sirloin cuts of commercial Australian beef obtained from three individuals (unknown strains) were used. To extract aldoses in beef, the sample was mixed with a reagent (water:methanol: chloroform = 1:2.5:1, V/V) and incubated at 37°C for 30 min. After centrifugation, the supernatants were freeze-dried. The derivatization method of beef sample was the same as described above. All experiments were performed in triplicate.

III. RESULTS AND DISCUSSION

We successfully identified 10 monosaccharides (ribose, ribitol, arabinose, xylose, xylitol, lyxose, allose, glucose, mannose and galactose) in a single GC/MS analysis (Figure 1). In this study, ribitol was added to each sample as internal standard and the standard curves of aldose were calculated. The curves of all analytes show good linearity within the concentration range of 1-20 µg/sample. In Figure 2, the representative curves of 3 monosaccharides (ribose, glucose and mannose) were summarized. Therefore, this analytical method used here might be sufficiently sensitive.

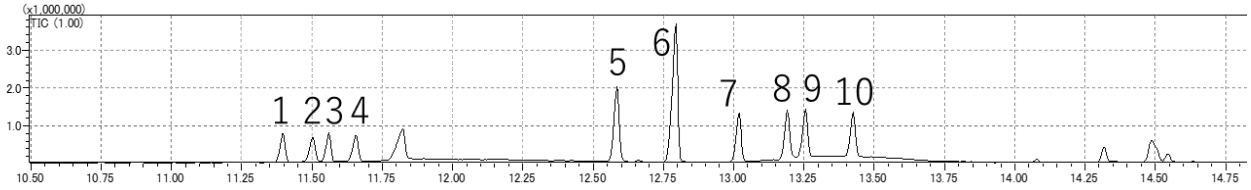

Figure 1. GC/MS chromatogram of aldononitrile acetate derivatized aldoses. Peak No.1: ribose, 2: lyxose, 3: arabinose, 4: xylose, 5: ribitol, 6: xylitol, 7: allose, 8: mannose, 9: glucose, 10: galactose.

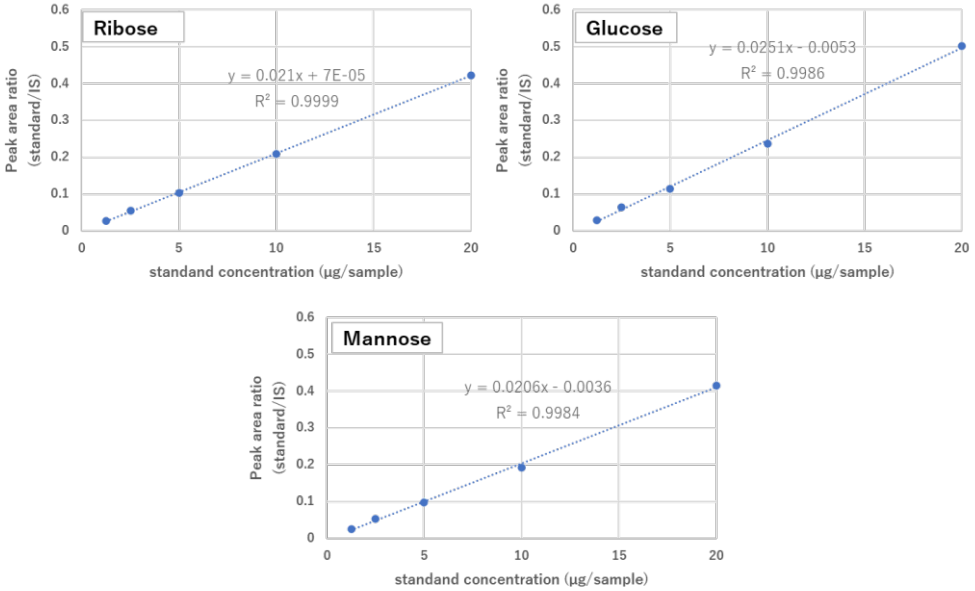

Figure 2. Standard curve of monosaccharides

Furthermore, the aldose concentrations of raw lean beef were measured by using AND. The beef sample contains 1.34 g/kg-beef of glucose, 0.38 g/kg of mannose and 0.17 g/kg of ribose. These results are similar to those of other report using a standard metabolomics approach [4].

IV. CONCLUSION

In the present study, our analytical methods using AND and GC/MS seems to be enough to quantify the concentration of aldoses in beef. We'll try to determine the aldoses in a heat-treated beef sample. Further studies are needed for clarifying which sugar compounds would be a candidate precursor of "retort beef aroma".

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INFLUENCE OF WEIGHT GAIN DURING REARING ON THE LONGISSIMUS MUSCLE AREA OF BEEF CATTLE

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I. INTRODUCTION

The intensification of beef cattle production systems is a trend that has been applied over the last few decades. This intensification aims to improve the productivity, sustainability and profitability of beef production. In tropical regions, improving productivity is proving to be an important tool for reducing methane gas emissions [1]. In tropical systems, the longest production phase is usually rearing, so it is crucial to increase the performance of cattle during this phase in order to reduce rearing time, thereby reducing the age at slaughter of cattle.

However, the consequences that improved performance during rearing has on finishing and the system as a whole are not yet fully understood. Increase weight gain in the rearing phase economically improves the production system as a whole [2]. In relation to finishing phase, it was observed that greater performance during rearing reduced the final weight, weight gain and intake of the cattle and did not alter their feed efficiency during finishing [3]. Weight gain during rearing did not affect subcutaneous fat deposition, but Longissimus muscle area (LMA) and carcass weight were higher in steers with better performance during rearing [4]. Therefore, the aim of the study was to evaluate the effect of weight gain during rearing on the LMA of Nellore cattle.

II. MATERIALS AND METHODS

This study was carried out at FZEA/USP on the Fernando Costa Campus and was approved by the Animal Research Ethics Committee (under protocol #1960131223) in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA). A total of 64 weaned Nellore steers were used in this study. Initially the animals had an average weight of 261.5±14.1kg and 7.1±0.4 months of age and were randomly distributed in a completely randomized design in four nutritional treatments (16 animals per treatment) based on weight gain during the rearing phase (8 months). The treatments were: T1 – Low gain (109.6 kg), T2 – Marginal gain (118.7 kg), T3 – Average gain (145.3 kg), and T4 – High gain (164.1 kg). The steers were housed in paddocks with *Urochloa brizantha* cv Marandu pastures and were supplemented for different performance.

The animals were weighed every 28 days in a handling center equipped with electronic scales Ultrasound carcass analyses were carried out at the beginning, mid and at the end of rearing to assess the LMA between the 12th and 13th ribs. This will be done using an Aloka SSD500 ultrasound equipped with a 17.2 cm linear transducer at a frequency of 3.5 MHz (Aloka Co. Ltd., Wallingford, CT, USA).

The data was analyzed using analysis of variance (ANOVA) in the “aov” function of statistical environment R (version 4.3.1). The residuals were tested for normality (Shapiro-Wilk test) and for homogeneity of variance (Levene’s test). The significant variables ($p \leq 0.05$) were submitted to pairwise mean comparisons using the Tukey-Kramer test. The treatment and sire were considered in the linear model. To assess the effect of the treatments throughout the time, a repeated measures over time analysis was carried out. To this analysis, we have included “Time” and “Time x Treatment” interaction in the linear model.

III. RESULTS AND DISCUSSION

There was a significant effect between treatments for LMA in rearing of Nellore cattle. In addition, the effect of time and the treatment x time interaction was significant for LMA. Treatments Average (T3) and High (T4) had higher LMA compared to Low (T1) and Marginal (T2) at the end of rearing and the same was observed for LMA gain during rearing (Table 1).

Table 1 – Longissimus muscle area (initial, mid, final and gain) of Nellore cattle of different performance during rearing.

Traits†	Treatments				*P-Values*		
	T1 Low	T2 Marginal	T3 Average	T4 High	Treatment	Time	Treatment x Time
Initial LMA, cm^2	49.9±3.7	50.8±3.9	51.4±5.1	54.1±3.3	0.07		
Mid LMA, cm^2	64.8±5.6	67.4±7.2	68.8±6.7	68.7±5.0	0.25	<0.001	<0.001
Final LMA, cm^2	70.8±4.8^a	70.6±3.5^a	76.1±5.8^b	84.3±6.5^c	<0.001		
Gain LMA, cm^2	20.9±5.2^a	19.7±4.3^a	24.67±5.2^b	30.2±6.1^c	<0.001		

†LMA = Longissimus muscle area. Superscript lowercase letters represent significant contrasts between treatments.

Increasing rearing performance is crucial in the search for more precocious animals and for the production of meat with greater tenderness. Tropical systems tend to have long rearing periods, which can make them less efficient financially and environmentally.

The results of this study showed that better gains during rearing increased LMA, which corroborates other studies [4,5]. However, this higher LMA in animals with better performance may be a reflection of the higher live weight of the animals at the end of rearing, and not necessarily greater LMA gain per unit of live weight [5]. Therefore, more studies are needed to better understand the effects of performance during rearing on muscle growth.

IV. CONCLUSION

Performance during rearing changes the Longissimus muscle area. More intensive systems with higher gains during rearing can increase muscle deposition in the carcass of *Bos indicus* beef cattle in tropical production systems.

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Glycogen supplementation in-vitro promotes pH decline in dark-cutting beef by reverting the muscle's metabolome towards normal postmortem muscle state

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I. INTRODUCTION:

The rate and extent of postmortem pH decline plays a significant role in determining meat color, with consumer-preferred characteristics such as a cherry bright-red color typically achieved at a postmortem muscle pH of approximately 5.6 (1). Deviations from this norm, particularly exceeding 5.8, are associated with muscle darkening, a well-documented phenomenon in dark-cutting beef (2). Previous research has demonstrated that dysregulated muscle glycogen metabolism pre-slaughter in dark-cutting beef phenotypes significantly affects substrate metabolism compromising glycolytic flux leading to less accumulation of lactic acid postmortem, thereby contributing to abnormal muscle pH (> 5.8). However, the underlying mechanisms have remained elusive. In this study, we aimed to examine the metabolic role of glycogen in regulating postmortem muscle darkening and pH decline in beef. We hypothesized that increasing glycogen levels in dark-cutting muscles could induce a metabolic shift capable of restoring normal postmortem metabolic programs. Our objective was to assess the impact of glycogen supplementation on muscle pH decline, as well as the activities of glycogen phosphorylase and lactate dehydrogenase enzymes, and the metabolite profiles of dark-cutting beef muscles.

II MATERIALS AND METHODS

Longismuss lumborum muscles from six bright red normal-pH and six dark-cutting beef loins (Institutional Meat Purchasing Specification #180, NAMP, 2002; grain-finished, spray chilled) sourced from A maturity carcasses were procured from a commercial beef processing facility. Upon procurement, samples were immediately transported on ice to Oklahoma State University Food and Agricultural product center where they were fabricated into steaks of 2.54 cm thick, then powdered in liquid nitrogen, and stored at -80 $^{\circ}$C until further analysis. Muscle glycolysis was stimulated as described (3) by homogenizing 100 mg of powdered *longissimus lumborum* muscles from twelve samples (n = 6 dark-cutting and n = 6 normal-pH beef) based on previous study (2) and incubating them in 1mL of an anaerobic buffer (10 mM Na_2HPO_4, 5 mM $MgCl_2$, 60 mM KCl, 5 mM ATP, 0.5 mM ADP, 0.5 mM NAD+, 25 mM carnosine, 30 mM creatine and 10 mM sodium acetate; pH 7.4), with or without glycogen at 0 and 10 mM. Normal-pH beef samples without glycogen served as a negative control. The pH of all treatments, was adjusted to the same point (pH = 7.0) using pH adjusting solutions. Subsequently, the reaction was monitored for 24 hours at room temperature (25 ºC). Post-incubation, pH, enzyme activities (glycogen phosphorylase and lactate dehydrogenase), and metabolite profiles were assessed. pH decline was measured using an Acument 50 pH meter, while enzyme activities were determined using standard enzyme assay kits from Abcam, and metabolomics profiling was conducted via a non-targeted gas chromatography mass spectrometry approach. Statistical analyses, including Two-way ANOVA for pH decline and enzyme activities, and pairwise t-tests for metabolomics data, were performed using GraphPad Prism V.10 and Metabolome Analyst V.6.0, respectively, with significance set at $\alpha = 0.05$.

III. RESULTS AND DISCUSION

Results showed that in vitro glycogen supplementation at 10 mM in dark-cutting beef led to a significant pH decline (pH = 5.87; $P < 0.05$) after 24 hours of incubation compared to both normal beef and untreated dark-cutting beef. This decline in pH decline following glycogen supplementation suggests substrate-mediated activation of enzymes involved with glycogen mobilization and utilization. Additionally, glycogen supplementation stimulated approximately a two-fold increase in glycogen phosphorylase (7.06 mUnit/mg tissue) and lactate dehydrogenase enzyme (61.87 pmol of NADH/min/µL) activities in dark-cutting samples. While lactate dehydrogenase activity was significantly lower in untreated dark-cutting compared to normal beef control ($P> 0.05$), glycogen phosphorylase activity exhibited a numerical increase in untreated dark-cutting beef. Metabolite profiling identified 132 metabolites, with 122 showing differential abundant across sample groups after 24 hours of incubation. Principle component analysis (PCA) revealed distinct clustering by treatments, with glycogen-supplemented dark-cutting treatments exhibiting separation from both untreated dark-cutting and normal-pH beef treatment groups. This indicates that glycogen is a key discriminative factor in the metabolites profiles. Pairwise comparison of metabolic profiles demonstrated differential abundance of 25 up-regulated and 31 down-regulated metabolites with > 2-fold change (FDR > 0.05) in glycogen-supplemented dark-cutting samples compared to untreated dark-cutting control while 22 up-regulated and 55 down-regulated metabolites were observed in comparison with normal-pH beef control samples (FDR > 0.05). Furthermore, examination of the specific differentially abundant metabolites between glycogen-supplemented dark-cutting samples and un treated controls demonstrated a strikingly greater abundance of glycolytic metabolites and reduced levels of tri carboxylic acid (TCA) cycle, amino acids, and nucleotide metabolites (FDR > 0.05). This metabolic reprograming resemblence normal beef metabolite profiles. Thus, our findings suggest that glycogen levels rather than enzyme abundances, may be the primary limiting factor in dark-cutting beef muscle pH decline.

IV. CONCLUSIONS

In this study we provide insights into the metabolic dynamics underlying dark-cutting beef, highlighting the crucial role of glycogen in modulating postmortem muscle pH decline and metabolic processes. The substantial decrease in pH observed upon glycogen-supplementation in dark-cutting beef illustrates the influence of substrate-mediated activation of key enzymes involved in glycogen utilization and mobilization. Moreover, the pronounced metabolic reprograming towards a profile resembling normal postmortem muscle, characterized by increased abundance of glycolytic metabolites and decreased levels of TCA cycle intermediates and amino acids, suggests that that pre-slaughter developmental events may contribute to substrate inherent inhibition mechanisms in dark-cutting beef. These findings imply that optimizing glycogen levels could represent a promising strategy for mitigating dark-cutting beef phenotypes and improving meat quality.

ACKNOWLEDGEMENTS

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INFLUENCE OF DIFFERENT ELECTRICAL STIMULATION TREATMENTS AT THE CUTTING ROOM ON QUALITY PARAMETERS OF DEBONED CHICKEN BREAST FILETS

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I. INTRODUCTION

As the demand for poultry meat as an affordable source of protein continues to increase, the industry is constantly challenged with meeting consumer needs. Maximizing production efficiency, through selection practices and improved nutritional programs have made possible to provide a affordable protein source to a variety of consumers [1]. To deal with the increasing production volume, it's became necessary to decrease the aging period, deboning early and chilling rapidly. However, these practices carry negative effects in regards to meat quality [2], introducing new obstacles to overcome. Electrical stimulation is a technology that may be used to improve meat tenderness of early deboned broilers, by accelerating the post mortem metabolism and rigor mortis development [3]. Therefore, this study aimed to observe the influence on physical-chemical meat quality parameters of nine different electrical stimulation treatments applied on deboned chicken breasts.

II. MATERIALS AND METHODS

Seventy two deboned chicken breasts from Cobb 500 male broilers (live weight ranging from 2.8 to 2.9 kg) were subjected to electrical stimulation at the cutting room. The birds were slaughtered at a line speed of 200 birds per minute and stimulated at 3 hours and 25 minutes *post mortem*, after reaching 4ºC. A prototype of a benchtop electrical stimulator was developed using a controlling font Fluxo LFX-500. It was applied 3 different electrical current intensities (500, 1000 and 2000 mA) during 3 different times (10, 20 and 30 s), totalizing 9 treatment groups, with 8 samples each. The left portion of each breast was stimulated, whereas the right portion was not, being kept as control. Subsequently the samples were frozen at -18ºC and thawed at 4ºC for 48h for the analysis.
The meat quality parameters evaluated were pH (benchtop pHmeter Kasvi K39-2014B), color according to CIE L*a*b* system (chroma meter Konica Minolta CR-400/410), water holding capacity (filter paper press method), drip loss, cooking loss, and Warner-Bratzler shear force (texturometer model TAXT2i, software Texture Expert V).
The results were subjected to analysis of variance and the difference between electrically stimulated and the respective non-electrically stimulated breasts was tested with the Student's t-test, at a significance level of 5% ($P < 0,05$).

III. RESULTS AND DISCUSSION

The meat quality parameters analysis (Table 1) indicated that there was no significant difference ($P > 0.05$) between all the 9 different electrical stimulation treatments evaluated and their respective controls. It's been demonstrated that electrical stimulation is a efficient method of reducing cold shortening and improving tenderness, however it is usually applied sooner, immediately after bleeding or after scalding [4]. Nevertheless, fillets stimulated after defeathering have also shown improvements [5]. Electrical stimulation induces a faster rate of glycolysis and rigor development in poultry, accelarating biochemical postmortem changes involved in the conversion of muscle to meat, therefore reducing the aging time [3]. As in the present study already deboned breasts were stimulated and soon after subjected to freezing, it's possible that the period was insufficient for the manifestation of signifficant effects.

Table 1 – Effect of current and time on meat quality parameters.

	Current (mA)	Time (s)	pH	L*	a*	b*	DL (%)	WHC (%)	CL (%)	WBSF (N/s)
T1	500	10	5,767	64.414	11.128	13.990	5.5	24.3	18.6	6.478
C1	-	-	5.808	64.098	11.338	13.815	4.6	25.3	17.1	6.415
T2	500	20	5,713	64.225	11.715	13.606	6.4	27.7	19.6	6.585
C2	-	-	5.795	64.663	11.687	14.535	5.7	26.5	27.9	6.486
T3	500	30	5.910	63.793	10.814	14.541	6.1	26.0	19.4	5.867
C3	-	-	5.852	64.087	11.043	15.257	5.4	25.2	19.5	6.119
T4	1000	10	5.815	64.319	11.615	15.151	6.3	26.2	17.3	6.689
C4	-	-	5.843	64.604	11.226	15.962	5.4	26.4	12.4	6.602
T5	1000	20	6.009	65.782	10.925	15.197	3.9	29.0	19.4	6.803
C5	-	-	5.936	65.500	11.100	15.054	3.9	30.6	18.6	6.475
T6	1000	30	6.052	62.948	11.556	13.235	4.1	25.1	20.6	10.215
C6	-	-	6.085	63.216	11.688	13.336	3.6	30.4	19.5	8.873
T7	2000	10	6.043	63.971	10.991	13.403	2.8	20.1	17.1	7.866
C7	-	-	6.076	62.811	11.690	12.875	2.5	25.1	15.7	7.691
T8	2000	20	5.908	63.464	11.467	12.942	5.3	22.7	22.0	8.755
C8	-	-	5.910	63.495	12.038	13.401	4.2	24.7	19.7	9.424
T9	2000	30	6.094	64.511	10.303	12.994	5.4	20.0	21.5	8.564
C9	-	-	6.083	64.257	10.396	13.595	4.0	26.5	19.8	8.204

[1] Abbreviations: DL, drip loss, WHC, water holding capacity, CL, cooking loss, WBSF, Warner-Bratzler shear force.
[T1-T9] Indicates the treatment means.
[C1-C9] Indicates respective control means.

IV. CONCLUSION

Electrical stimulation on deboned broiler breasts at the cutting room didn't show statistically significant differences ($P > 0.05$) in quality traits in comparison to non-stimulated filets, regardless of the current and duration of the application. It is possibly due to the moment in which the samples were stimulated. Therefore, it's necessary to determine until when it is possible to obtain significant improvements on meat quality using electrical stimulation, as well as the most efficient moments of application during poultry meat processing.

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VASCULAR RINSING LAMB CARCASSES WITH CALCIUM CHLORIDE IN COMBINATION WITH ELECTRICAL STIMULATION CAUSES PROTEOLYTIC CHANGES ASSOCIATED WITH MEAT TENDERNESS

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I. INTRODUCTION

This study aimed to determine the impact of adding calcium chloride to the carcass vascular rinse solution (Rinse & Chill®, MPSC Inc.) in combination with electric stimulation on lamb meat tenderness. Calcium infusion in meat carcasses activates μ-calpain and m-calpain, which enhances proteolysis and improves meat tenderness. In a previous study[1], calcium was infused with a manual syringe pump and did not include electric stimulation or Rinse & Chill. The use of Rinse & Chill technology improves meat tenderness by accelerating a rapid pH decline early post-mortem[2]. This technology provides a commercial means to deliver calcium to prerigor muscles to impact proteolytic changes. We hypothesized that lamb carcasses vascularly rinsed with the Rinse & Chill solution containing the addition of calcium chloride coupled with electric stimulation would cause more proteolysis than the standard Rinse & Chill solution.

II. MATERIALS AND METHODS

Lambs (total n=13) of market age were randomly assigned to three different carcass vascular rinsing postmortem methods (treatment) which included: 1) RC (Rinse & Chill solution; 98.5% water; balance: glucose, polyphosphates, maltose; 10% live weight), 2) CA (RC + 0.3 M calcium chloride), and 3) ES-CA (electrical stimulation followed by CA). Lambs were humanely stunned and exsanguinated prior to carcass treatment. A cannula was inserted into the heart to infuse the test solutions through the vascular system. For ES-CA, carcasses were electrically stimulated for two consecutive 30-second periods (800 milliamps) before the rinse. Carcasses were chilled (3 ℃, 24 h) before fabrication. Longissimus dorsi (LD), semimembranosus (SM), and triceps brachii (TB) muscles were collected, vacuum packaged, and aged (3, 7 days). Muscle calcium was determined (ICP-OES, AOAC 985.01). Myofibril fragmentation index (MFI) was used to assess proteolytic changes[3]. MFI was calculated by multiplying absorbance (540 nm, 0.5 mg/mL protein) by 200. Warner-Bratzler Shear (WBS) was determined on strips from cooked chops (68.3 ℃, internal; 1-cm x 1-cm, strips). Data were analyzed as a split plot design (carcass treatment, whole plot factor; muscle, split-plot factor).

III. RESULTS AND DISCUSSION

CA and ES-CA increased the amount of calcium in the muscles (Table 1). MFI mean was largest for RC. CA and ES-CA were not different from one another in the LD and TB (Table 1). Addition of calcium was expected to enhance proteolysis associated with calpains thus producing a greater MFI than RC. Microscopically the supernatant of CA and ES-CA treatments appeared to contain more, very small myofibrillar fragments than RC. As such these fragments were not retained in the pellet used to determine MFI. Calcium-containing treatments resulted in shorter sarcomeres and visually apparent muscle degradative changes (Figure 1). For LD chops, treatments containing

calcium had lower WBS values (P<0.05, 22.3 N for CA, 20.9 N for ES-CA) than RC (29.6 N). For SM, WBS was lower for ES-CA than RC (30.1, 36.5 N; respectively).

Table 1 – Least square means[1] for calcium content and myofibril fragmentation index among treatments and muscles in lambs.

	Calcium (mg/kg)			MFI		
Treatment	LD	TB	SM	LD	TB	SM
RC	50.6^{e}	50.2^{e}	53.4^{e}	136.1^{a}	112.9^{b}	105.1^{b}
CA	699.4^{ab}	247.8^{de}	421.4^{cd}	63.0^{d}	50.1^{d}	82.8^{c}
ES-CA	915.8^{a}	415.0^{cd}	541.9^{bc}	51.0^{d}	52.8^{d}	60.8^{d}

[1]Means with unlike superscript letters within calcium or MFI are different (P<0.05; treatment * muscle. Calcium S.E.= 102.7; MFI S.E.= 6.61).

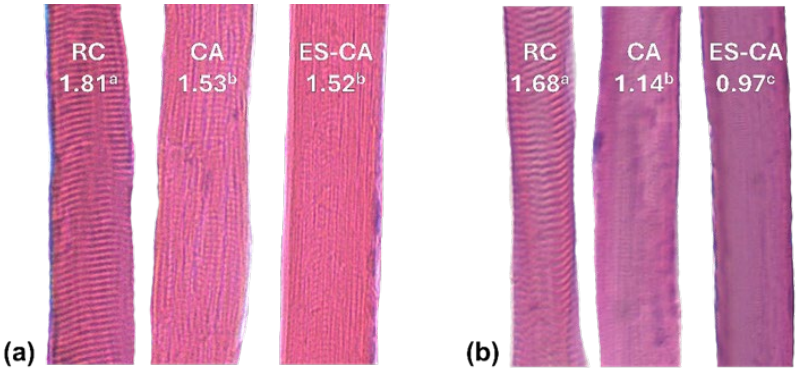

Figure 1. Effect of various carcass treatments on myofiber structure (eosin and hematoxylin stained, 40X). a) Longissimus dorsi. b) Semimembranosus. Sarcomere lengths (microns, means within a muscle with unlike letters are different, P<0.05, LD S.E. 0.0274; SM S.E. 0.0486).

IV. CONCLUSION

Based on microscopic assessment and instrumental shear determination, inclusion of calcium in the Rinse & Chill® solution may offer packers the opportunity to increase tenderness. However, the MFI method might not be reliable for assessing tenderness in highly proteolytically tenderized meats. Analysis of the amount of myofibrillar proteins at each MFI step warrants further investigation.

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Effect of aging and intramuscular fat grade on WBsf in Longissimus Thoracis Muscle of Hanwoo

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I. INTRODUCTION

The tenderness of beef is a crucial factor influencing consumer satisfaction and overall dining experience [1]. Among the various factors that affect beef tenderness, aging and intramuscular fat grade play significant roles [2]. Intramuscular fat, commonly referred to as marbling, contributes to the sensory attributes of beef, including tenderness, juiciness, and flavor [3]. On the other hand, aging, a process of controlled enzymatic breakdown of muscle fibers post-slaughter, is recognized for its ability to enhance beef tenderness by allowing natural tenderization mechanisms to take place [2]. The aim of this study was to compare the relative impact of aging and intramuscular fat grade on the Warner-Bratzler shear force (WBsf) in the Longissimus thoracis (LT) muscle of Hanwoo.

II. MATERIALS AND METHODS

From 2007 to 2020, data on Hanwoo beef analyzed at Jeonbuk National University and the National Institute of Animal Science were collected. The analysis results for the LT muscle from a total of 423 heads (32 bulls, 75 cows, 12 heifers and 304 steers) generated across four projects were utilized. All animals were conventionally raised in feedlots and slaughtered according to the regulations set forth by the Korea Animal Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs for beef slaughter at commercial abattoirs. And then immediately moved to a chilling room and stored at 4 °C, after 24 h in the chilling room, all carcasses were graded according to the Korean Beef Carcass Grading System included intramuscular fat grade [4]. Aging was initiated 24 hours post-slaughter, designated as Day 0 of aging. Shear force measurements were conducted using a Warner-Bratzler blade. Meat blocks were heated in a water bath until the core temperature reached 70°C, then cooled in running water for 30 minutes. Cores(1.25cm) parallel to muscle fibers were took, and shear force was measured using an Instron Universal Testing Machine (Model 3342; Instron Corporation, Norwood, MA, USA) The statistical analysis was conducted using IBM SPSS Statistics (version 27.0, SPSS Inc., Chicago, IL, USA). The comparison of the impact of aging and intramuscular fat level on shear force was conducted using multiple regression analysis.

III. RESULTS AND DISCUSSION

The results of the multiple linear regression analysis conducted to investigate the impact of aging and intramuscular fat level on shear force in LT muscle of Hanwoo are presented in Table 1. In the Hanwoo LT muscle, aging had a significant effect on shear force with β=-0.019 ($p<0.001$), and intramuscular fat grade also had a significant effect with β=-0.083 ($p<0.001$).
The relative impact of aging and intramuscular fat grade on shear force value was compared through the standardized coefficients β values.

The standardized coefficients β values for aging and intramuscular fat level were -0.311 (p<0.001) and -0.286 (p<0.001), respectively, indicating that aging has a relatively higher impact on shear force compared to intramuscular fat grade.

Table 1 – Comparison of the impact of aging and intramuscular fat grade on shear force in the longissimus thoracis Muscle of Hanwoo.

Variations	Unstandardized coefficient		standardization coefficient	t(p)	TOL	VIF
	β	SE	β			
(Coefficient)	4.366	0.078		55.928		
Aging days	-0.019	0.003	-0.311	-6.784***	0.896	1.116
Intramuscular-fat grade	-0.083	0.013	-0.286	-0.263***	0.896	1.116
F(p)			32.228***			
Adj.R^2			0.117			
Durbin-Watwon			0.628			

***P<0.001

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Elite Dairy Beef – A pathway for male dairy calves into the premium beef market

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I. INTRODUCTION

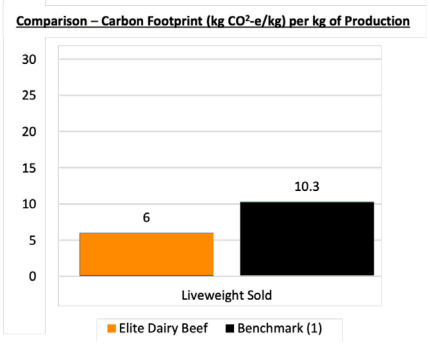
The Elite Dairy Beef program is based on consumer eating experience from dairy beef raised under a unique tightly controlled nutritional program. The program is aimed at changing industry perception of "dairy beef" from the traditional lower quality manufacturing image to a high value premium product that justifies the raising of male dairy calves. A requirement for cattle marketed under this brand requires animals to be antibiotic, hormone and ionophore free. Typically, many dairy beef programs have seen high incidences of disease and defects at slaughter in dairy cattle fed on an accelerated pathway.

II. MATERIALS AND METHODS

The nutritional program was initially developed in Spain and is widely used in Europe and the UK in the premium beef programs. A locally adapted program utilising key ingredients from Spain in conjunction with local sourcing has since been tested and proven in Australia. The milk replacer and rations are of extremely high quality and specifically targeted at superior early life nutrition to rapidly develop the calf immune system and rumen function, thereby maximising health and avoiding the use of antibiotics. Contrary to conventional rearing systems, this system is based on a low milk replacer intake and immediate concentrate consumption from birth. The critical and interlinked aims are to avoid negative energy balance, optimise gut health, and fast-track rumen development.
Calves are purchased at 5 days of age and transported to a rearer. They have ad lib access to concentrate Quickstart, plus specialised milk powder InzarMilk, fed at 2L twice a day for 3-4 weeks. After 2 weeks, calves transition onto a grower ration Papincalf enabling early weaning of the calf. This ration is fed until 14 weeks after which cattle are transitioned onto the final ration Econbeef until slaughter.

III. RESULTS AND DISCUSSION

A preliminary carbon results for the supply chain are displayed below. The Elite Dairy Beef pathway results in 50% lower carbon when compared to traditional beef (Figure 1 and 2).



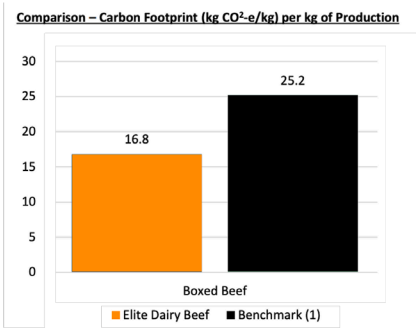


Figure 1 and 2: Elite dairy beef pathways carbon footprint liveweight and boxed beef effect relative to that of traditional beef.

The initial results for processed carcasses are presented in Tables 1 & 2 below for both animal performance and carcass characteristics. These cattle are currently sitting in the top 10% for eating quality of all cattle fed in Australia.

Table 1 and 2: Elite dairy beef animal performance and carcass results from currently processed cattle (n = 1913)

Animal Data	Average	Min	Max
Exit Weight	558.9	348	718
Finisher ADG	1.32	0.51	2
Lifetime ADG	1.19	0.9	1.7
Finisher FCE	5.27	2.5	7.1
Lifetime FCE	4.96	2.8	7.6
Slaughter Age	16	9	20

Carcass Traits	Average	Min	Max
HSCW	286.7	174	372.5
Dressing %	54%	40%	74%
Ossification	135	100	170
Hump	55	5	260
MSA Marbling	370	40	1050
Ribfat	8.5	3	27
Ultimate pH	5.53	5.04	6.5
Eye Muscle Area	64	20	89
MSA Index	62.8	56.04	72.11

Animal health disease and defect results at slaughter are only recorded against 6.1% of the current population, with the highest occurrence in rumen abscesses. This gives a good indication that these calves have been programmed differently during the early phases for early immune and rumen development.

IV. CONCLUSION

Cattle are finishing slightly older than initially expected, however this is reducing with improvements to the system. Cattle are achieving liveweights of 500-550kgs and carcass weights of 280-300kgs. Cattle carry a positive carbon story, with the calf primarily offset by the cow coupled with a highly efficient animal capable of good conversion and fast finishing times. Finally, animal health disease and defect data does not currently indicate that any additional pressures placed on the animal during the accelerated feeding program are adversely affecting the animal internally.

COMPARISON OF DENTAL CARCASS MATURITY IN NELLORE BULLS: EFFECTS ON CARCASS AND MEAT QUALITY TRAITS

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I. INTRODUCTION

Brazilian slaughterhouses use the number of permanent incisor (PI) teeth to classify beef carcasses, which indicates the age at slaughter [1]. However, the number of PI teeth does not directly reflect the physiological maturity, with little association with meat quality traits such as intramuscular fat, color, and tenderness [2]. Therefore, United States and Australia use the ossification score (OS) to indicate physiological maturity and its impacts on beef quality [3,4]. Thus, the objective was to evaluate how age (measured by PI teeth) impact the carcass and meat quality traits of Nelore bulls and its association with OS.

II. MATERIALS AND METHODS

Nellore bulls (*Bos indicus*) with average final body weight of 550 ± 11 kg were used. At slaughter, following head inspection, the number of PI was recorded for each animal. Subsequently, carcasses were randomly selected per dentition group, totaling 90 carcasses grouped in two categories (6 PI [n=45] and 8 PI [n=45]). After 24h chilling (2 to 4 °C), all carcasses were evaluated for OS, following the Australian model [5]. Additionally, hot carcass weight (HCW), ribeye area (REA), backfat thickness (BFT), and marbling score (MAR) were assessed. At deboning, *Longissimus thoracis* samples between the 11th and 13th ribs of the left half-carcass were collected. Two samples (2.54 cm thickness) from each experimental group were vacuum- packed and wet-aged for 21 and 28 days (1 to 2 °C) and then frozen (-20 °C). Initially, the samples were thawed (1 to 2 °C for 24h) and exposed to oxygen for 30 minutes at 2 °C (*blooming*). The meat pH, water- holding capacity (WHC), and color parameters (*L*, a*, b*, Chroma,* and *Hue*) were measured according to described procedures [6,7]. Finally, Warner-Bratzler shear force (WBSF) and cooking losses – CL (divided in evaporation [EL] and drip [DL]) were obtained [8,9]. Data of carcass (HCW, BFT, REA, OS, and MAR) and meat quality traits (pH, WHC, *L*, a*, b*, Chroma, Hue*, WBSF, CL, EL, and DL) were compared using analysis of variance (ANOVA). The animal (carcass) was considered the experimental unit, and PI number (treatment) was used as a fixed effect in the statistical model. Differences were considered significant when $P<0.05$. The relationships between variables were also studied through principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) using R software (v.4.1.2).

III. RESULTS AND DISCUSSION

Greater OS and REA ($P<0.05$) were observed in animals with 8 compared to 6 PI (Figure 1), while HCW, BFT and MAR were similar. Moreover, PI teeth impaired ($P<0.05$) meat color at 28 days of aging, reducing *b** and *Hue* (Table 1). Additionally, DL was lower ($P<0.05$) in 8 PI group, whereas other meat quality traits were similar. Both variables OS and DL affects data variability at 21 days (Figure 2A), whereas OS, WHC, *b**, and *Hue* were the most important variables at 28 days of aging (Figure 2B). Relationships between OS and WHC, as well as between pH and color parameters (*b** and *Hue*), help explain the separation of PI groups projected in the multivariate space (27% of accumulated variance) showing why these animals had differences in meat quality. Particularly, animals with 8 versus 6 PI were divergent for OS, WHC, *b** and *Hue*, suggesting differences in musculosity, chemical composition or lipid content, as observed [10]. However, additional biochemical assays are necessary to confirm this hypothesis.

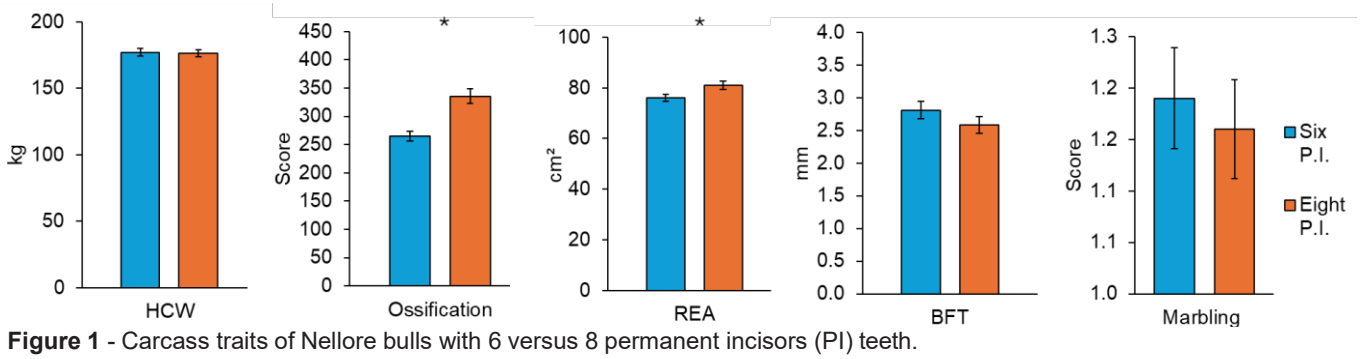

Figure 1 - Carcass traits of Nellore bulls with 6 versus 8 permanent incisors (PI) teeth.
Variables: HCW = Hot carcass weight; REA = Rib eye area; BFT= Backfat thickness. *Significance $P<0.05$.

Table 1. Meat quality traits of Nellore bulls with 6 versus 8 permanent incisors (PI) teeth.

Variables[1]	PI teeth 6	8	*P*-value	PI teeth 6	8	*P*-value
	21 days of aging			28 days of aging		
pH	5.53 ± 0.1	5.51 ± 0.2	0.471	5.27 ± 0.2	5.32 ± 0.1	0.106
*L**	38.02 ± 2.2	37.53 ± 3.6	0.394	40.78 ± 3.5	41.83 ± 3.5	0.161
*a**	17.64 ± 2.0	17.71 ± 2.4	0.888	18.25 ± 2.6	18.23 ± 1.7	0.770
*b**	7.85 ± 1.4	8.03 ± 1.6	0.579	9.10 ± 1.5	8.42 ± 1.6	0.049
Chroma	19.34 ± 2.2	19.49 ± 2.6	0.773	20.44 ± 2.7	20.11 ± 2.1	0.532
Hue	23.97 ± 3.5	24.35 ± 4.3	0.711	26.59 ± 3.8	24.58 ± 3.3	0.010
WBSF	3.66 ± 0.7	3.66 ± 0.8	0.768	3.46 ± 0.7	3.45 ± 0.6	0.879
CL	31.47 ± 5.0	31.00 ± 4.8	0.642	33.34 ± 5.0	34.61 ± 11.0	0.850
EL	28.20 ± 6.0	27.86 ± 5.7	0.786	29.19 ± 5.8	31.89 ± 11.7	0.189
DL	3.26 ± 2.3	3.13 ± 2.6	0.805	4.15 ± 2.7	2.71 ± 1.8	0.004
WHC	61.28 ± 2.5	61.13 ± 2.3	0.775	59.52 ± 3.0	58.24 ± 3.7	0.082

[1]pH = meat pH, *L*:* lightness, *a**: redness, *b*:* yellowness, WBSF: Warner-Bratzler shear force, CL: cooking losses, EL: evaporation loss, DL: drip loss, WHC: Water-holding capacity.

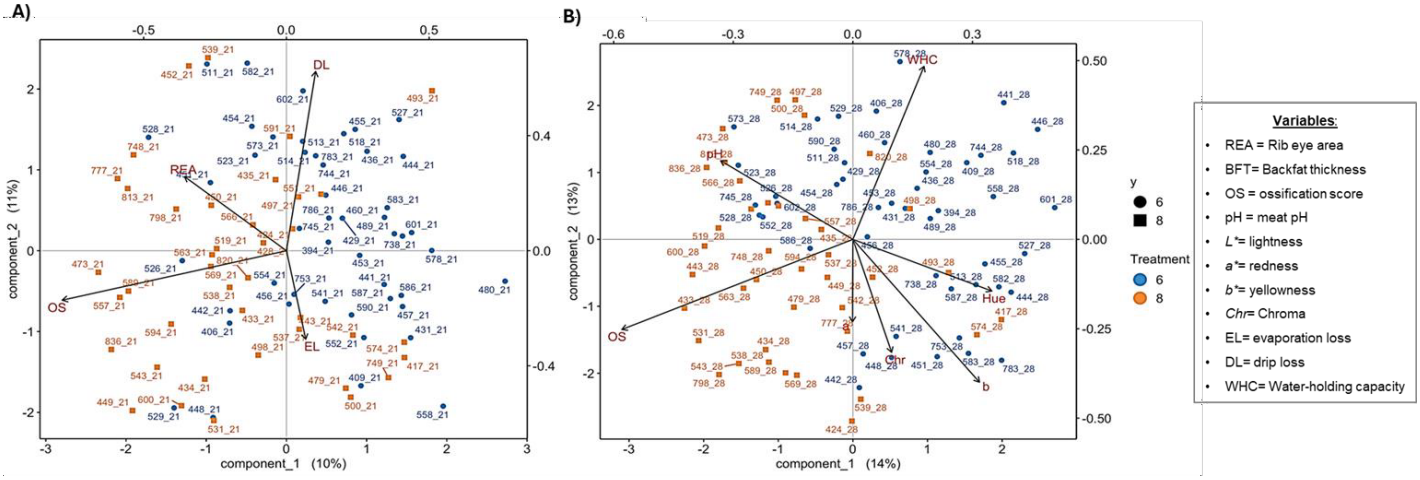

Figure 2. Principal component analysis and partial least squares-discriminant analysis of carcass and meat quality traits of Nellore bulls with 6 versus 8 permanent incisors (PI) teeth. Meat aged for 21 (**A**) and 28 (**B**) days.

IV. CONCLUSION

Nellore bulls slaughtered with 6 or 8 PI teeth differ in terms of OS and REA but are similar regarding meat tenderness (WBSF) and pH. However, meat color (yellowness and *Hue*) of 8 PI group was impaired at 28 days of aging, while drip losses decrease compared to 6 PI group.

ACKNOWLEDGEMENTS

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The PI3K-Akt signaling pathway plays an important role in the differentiation of adipocytes promoting the adipogenesis in crossbred calves supplemented with vitamin A

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I. INTRODUCTION

In Brazil, with the increased use of crossbreeding with *Bos taurus* in meat production, the amount of intramuscular fat (IMF) has received greater attention from consumers and meat industry, since zebu or *Bos indicus* animals (predominant in the country) produce meat with little or even no marbling [1]. With higher levels of IMF content (or marbling), the tenderness, juiciness, and flavor of the meat can be improved [2]. The vitamin A supplementation in young cattle has drawn interest due to its capacity to modulate adipogenesis, as evidenced [3]. This nutritional intervention holds potential for enhancing IMF content. The objective of this study is to identify the signaling pathways of adipogenesis in crossbred *Bos taurus* × *Bos indicus* calves supplemented with vitamin A and finished in feedlot.

II. MATERIALS AND METHODS

Thirty-four F1 Montana × Nellore male calves were used (17 without vitamin A [Control] and 17 with vitamin A [VitA]). At birth, VitA calves received a intramuscular dose of 300,000 IU of Vitamin A, while Control calves received a placebo. At 270 days all animals were weaned and feedlot finished for 180 days. Experimental groups were slaughtered with final body weights of 398 and 415 kg ± 11 kg - Control and VitA, respectively. The intramuscular fat (IMF) content of meat was assessed by infrared spectroscopy in a FoodScan™ equipment (FOSS, Denmark). Total RNA from the *Longissimus thoracis* (LT) samples (n=6/ group) was extracted individually from 100 mg of LT using TRIzol® (Life Technologies, USA), according to the manufacturer's instructions, and analyzed on the Bioanalyzer 2100® (Agilent, USA). The RNA libraries for each sample were prepared using the TruSeq RNA Sample Preparation Kit (Illumina, USA) from 2 µg of total RNA, according to manufacturer's instructions. Finally, sequencing was carried out on the Illumina NextSeq550® (Illumina, USA) in order to produce paired-end reads of 100 bp. The genes with differential expression as a function of VitA treatment were identified according to their biological function and subsequently categorized into functional groups using *enrichR* and *ClusterProfiler* packages in R.

III. RESULTS AND DISCUSSION

Differences ($P < 0.05$) were found in IMF content of meat, with the Control group exhibiting 2.57% while the VitA group showed 4.10% (SEM = 0.28). Such differences in IMF content can be explained by adipogenic and lipogenic pathways upregulated in response to vitamin A (Table 1; and Figure 1). Adipogenesis involves a cascade of transcription factors that regulate the expression of genes involved in adipocyte development. For example, the phosphatidylinositol 3-kinase (PI3K)-Akt pathway plays an important role in adipocyte differentiation, promoting adipogenesis through the phosphorylation of certain substrates [4]. Studies have investigated the association of the FoxO, PI3K-Akt and cAMP pathways as regulators of glycolytic [5] and lipid metabolism [6], which help to explain greater IMF observed in animals from VitA treatment in the current study. However, the regulation mechanisms of some genes expressed in the PI3-Akt pathway for IMF deposition in cattle are still poorly understood.

Table 1. Absolute and relative numbers of skeletal muscle genes of Montana × Nellore male calves.

Contrast[1]	Total of DEGs[2]	Expression[3]	Absolute	Relative (%)
VitA vs. Control	165	Down	106	0.75
		NS	13.911	98.82
		Up	59	0.42

[1] VitA = calves suplemented with 300,000 IU of Vitamin A; Control = non vitamin A suplemented;
[2] DEGs = diferencially expressed genes obtained by the likelihood ratio test; A $\log_2$ fold change of 0.5 and significance adjusted to false discovery rate [FDR] < 0.05 were adopted to identify DEGs.
[3] Down = down-regulated; Up = up-regulated; NS = non-significant

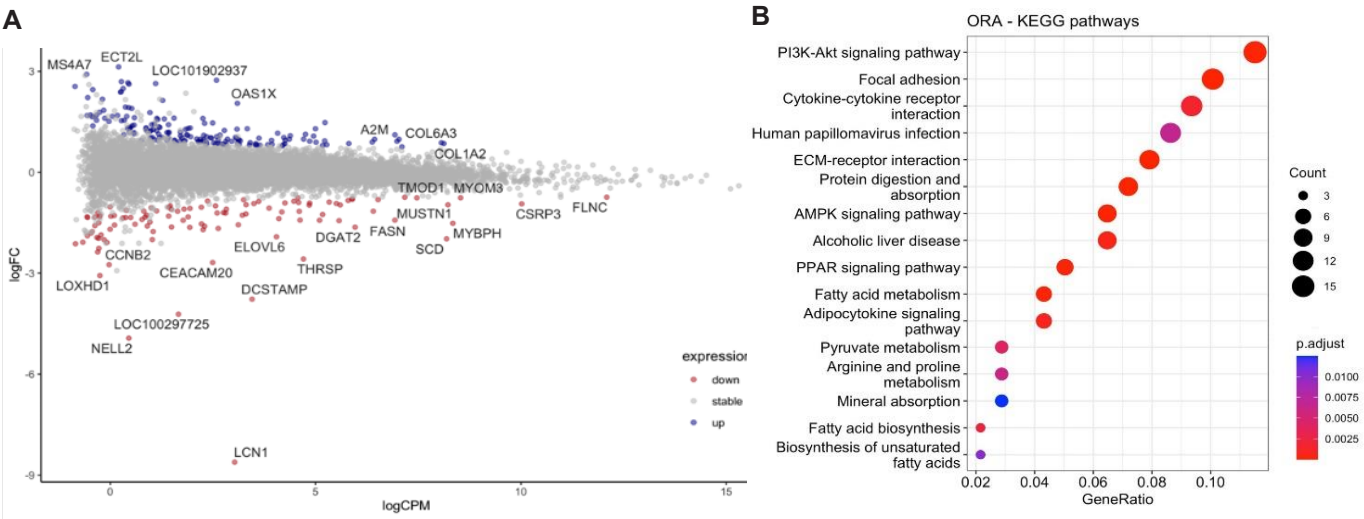

Figure 1. Differentially expressed genes (DEG; *P*-value > 0.05 and logFC > 0.5). Up-regulated in blue and down-regulated in red (**A**). Enriched metabolic pathways (KEEG) in VitA versus Control treatment (**B**).

In agreement with previous studies [3, 7, 8], upregulating *PCK2* and *PIK3R3* genes in lipid metabolism pathways increased IMF content. Notably, the present study reveals changes in these genes associated with the PI3-Akt pathway following VitA treatment. Specifically, the upregulation of *PCK2* potentially disrupts the balance of energy metabolism pathways, thus impacting lipid metabolism dynamics. Furthermore, greater expression of *PIK3R3* likely enhances PI3K activity, thereby modulating downstream signaling cascades involved in lipid metabolism and cellular growth. These findings shed light on the molecular mechanisms driving changes in IMF content and suggest a potential regulatory role for VitA in cattle lipid metabolism.

IV. CONCLUSION

The differences observed in the deposition of IMF in the LT muscle of Montana × Nellore crossbred calves were related to genes and pathways of lipid metabolism. Our study presents novel evidence suggesting that the PI3K-Akt signaling pathway may serve as a crucial regulator in adipocyte differentiation, thereby promoting adipogenesis and lipogenesis in crossbred calves receiving vitamin A supplementation (intramuscular dose) at birth.

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DIGESTIBILITY OF PROTEIN HYDROLYSATES FROM GAME MEAT

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I. INTRODUCTION

The game meat industry is growing in South Africa, thus expanding the range of protein sources. This study aimed to identify the most digestible protein fraction from springbok loin and investigate the techno-functional properties of these protein fractions. Protein hydrolysis results in active and inactive peptides. Peptides can improve functionality and produce bioactive peptides with health benefits. Several bioactive peptides impart antidiabetic, antioxidant, and antihypertensive properties. Bioactive peptides produced from game meat protein have the potential to help reduce the onset of diet-related non-communicable diseases, such as heart disease, stroke, and type 2 diabetes, when consumed in food products. This study was conducted to determine the effect of enzymatic hydrolysis (using Alcalase®) on the in vitro digestibility and techno-functional properties of whole protein and its fractions (sarcoplasmic and myofibrillar protein).

II. MATERIALS AND METHODS

Springbok (Antidorcas marsupialis) loins (a total of three) were purchased from Delicious Trading 5 (Pty) Ltd (Gauteng, South Africa). The loins were each, separately ground using a food processor. The blended meat was used immediately for protein extraction. The sarcoplasmic and myofibrillar proteins were extracted following a method described by (1). The blended springbok meat was homogenized with 0.03 M phosphate buffer (pH7), 1:10 ratio, for 30 seconds using a Waring blender. Half of the whole protein (WP) protein was divided into airtight containers and frozen at -20°C until analysis. Protein hydrolysates were prepared using the Alcalase® enzyme. For whole protein: 139.75mg alcalase was added to 10ml protein; sarcoplasmic protein: 41.55mg alcalase was added to 10ml protein; myofibrillar protein: 12.78mg alcalase was added to 10ml protein. The hydrolysates' degree of hydrolysis, in vitro protein digestibility and functional properties were also determined. An Analysis of Variance (ANOVA) was performed on the data from the different protein fractions and compared at $P \leq 0.05$ using Fisher's least significant difference (LSD) test following the general linear model (GLM) procedure.

III. RESULTS AND DISCUSSION

Table 1 shows the protein digestibility (g/100g), and degree of hydrolysis (%) of the hydrolysates from different protein fractions. Figure 1 shows the SDS-PAGE bands from the whole protein and the alcalase hydrolysed Springbok protein fractions. The protein digestibility of the different extracted fractions (sarcoplasmic and myofibrillar) tends to decrease ($p < 0.05$) when compared to the whole protein (2). This may be due to concentration or agglumatioation due to similarity of the fractional composition restricting enzyme access to binding sites. The protein digestibility likely decreases with each extraction. The estimated protein concentrations for the hydrolysed proteins are lower than their corresponding unhydrolyzed proteins, indicating that protein is lost during hydrolysis.

Table 1 – Protein digestibility of all the protein samples and degree of hydrolysis (%DH) of hydrolysates.

Protein Sample	**Digestibility (g/100g protein)**	**%DH**
Whole Protein	82.312 ±0.26^{c}	-

Sarcoplasmic	74.43 ±0.13[b]	-
Myofibrillar	72.9 ±0.77[b]	-
Hydrolysed Whole Protein	67.38 ±0.38[a]	1.74±0.07[a]
Hydrolysed Sarcoplasmic	66.75 ±0.26[a]	2.30±0.10[b]
Hydrolysed Myofibrillar	67.20 ±0.13[a]	2.09±0.13[bc]

For each column, mean values with different alphabets are significantly different ($p < 0.05$).

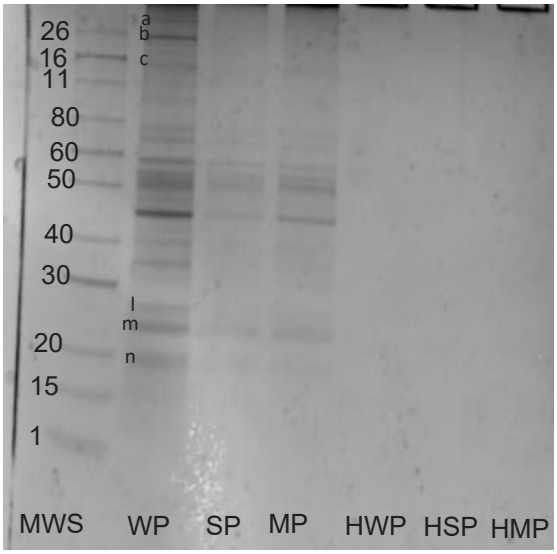

Figure 1. SDS-PAGE gel staining of hydrolysed and hydrolysed proteins

Figure 1: SDS-PAGE gel staining of hydrolysed and hydrolysed proteins. The molecular weight standard bands range between 260-10kDA. MWS= Molecular weight standard, WP= Whole protein, SP= Sarcoplasmic protein, MP= Myofibrillar protein, HWP= Hydrolysed whole protein, HSP= Hydrolysed sarcoplasmic protein, HMP= Hydrolysed myofibrillar protein. Letter A labels the bands of different molecular weights in the protein extracts.

IV. CONCLUSION

The study was conducted to determine the effect of enzymatic hydrolysis (using Alcalase®) on the *in vitro* digestibility and techno-functional properties of whole protein and its fractions (sarcoplasmic and myofibrillar protein). In terms of *in vitro* protein digestibility, it was found that enzymatic hydrolysis decreased the digestibility of whole protein and its fractions. Whole protein was the most digestible protein, which should be considered when finding novel ways of utilizing game meat proteins.

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From sub-zero to cryo-freezing: detectability of beef quality differences among different freezing temperatures

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I. INTRODUCTION

Food safety agencies in the European Union require previously frozen, unprocessed meat to be labeled as 'defrosted'. While different test methods for authentication of defrosted versus fresh meat have been suggested, no standard method for detection of freeze damage has been established yet [1]. A key challenge is that suitable test methods should reliably detect freeze–-related damage in meat over a wide range of temperatures that are currently used in the meat sector. This includes freezing at higher sub-zero temperatures (around –5°C) that do not fulfill EU regulations for the 'quick-frozen' label. In contrast, most studies on assessing improved freezing by novel technologies have typically focused on lower temperature ranges covered by the 'quick-frozen' label (–18°C and below). We could previously show that different spectroscopic methods only could resolve larger freezing temperature differences among ca. –25°C and cryo-freezing (below –196°C), while cryo-electron-microscopy also detected differences between –25°C and –35°C freezing. Using beef samples, we here assess the suitability of two additional test assays and ask if they allow detecting differences among four freezing temperatures between –4°C and –80°C. To this end we use an established enzyme assay that analyses the activity of a mitochondrial enzyme (HADH) that is released by freeze–related damage. In addition, we have adopted a protocol that allows preserving the 3-D matrix, including ice-crystal cavities, of frozen meat. Lastly, using the HADH-based enzyme assay, we also ask if higher temperature sub-zero freezing at –4°C can be distinguished from refrigerated only beef.

II. MATERIALS AND METHODS

We obtained beef (*Bos taurus*) samples 1d postmortem (semimembranosus, N=24 individuals). Five replicate samples (5x5x4cm^3) from each individual were subjected to the different treatments: 2°C (chilled only), –4°C, –14°C, –20°C, –80°C. Post-treatment samples were collected after 21d, including two days of defrosting for enzyme activity analyzes. Samples for 'frozen state' analyses were 'cryo-fixated' and stored at –80°C. Preserving the frozen state, i.e., cavities from ice crystallization, was done by adopting common freeze-dry protocols for microscopy. Briefly, cryo-stored samples were initially fixated with a 1% glutaraldehyde in acetone solution (also –80°C), then temperature equilibrated at (–20°C), and finally dried at room temperature using a drying chamber to evaporate acetone. With microscopy we observed that different freezing caused marked differences in cavity size, causing samples to appear darker respectively lighter. To assess such differences in lightness we used a Konica Minolta Chroma Meter CR-400 (Konica Minolta Sensing INC, Japan) to record the *L** parameter. For HADH testing, the drip loss was collected and diluted 1:5 in phosphate buffer (0.1M, pH6.0). The measurement of HADH enzyme activity followed Gottesmann et al. [2]. In brief, to one volume of diluted drip loss we added 2V EDTA solution (34,4mM), 2V NADH solution (7,5mM) and 22V of the phosphate buffer. Addition of 3V of acetoacetyl-CoA solution (5,9mM) started the reaction.

The reduction of NADH was measured at 340nm with a photospectrometer, and activity was calculated in U/ml using the molar extinction coefficient of NADH of 6.3 [l×mmol–1×cm–1]. Statistics were calculated using a one-way ANOVA for overall treatment effects and Tukey's test for pairwise testing.

III. RESULTS AND DISCUSSION

Both, L* values of the 'frozen-state' samples (Fig. 1A) and HADH activity (Fig. 1B) indicate marked effects of freezing temperatures, with –80°C showing significant differences compared to other temperatures. However, while L* values at –4°C were not distinguishable from –14°C and –20°C, the HADH assay showed such difference. The HADH assay also allowed a direct comparison with chilled controls (2°C). We found that the HADH assay could separate all lower temperature freeze treatments (–80/–20/–14°C), but not –4°C sub-zero freezing, from the chilled group (2°C, Fig. 1B)

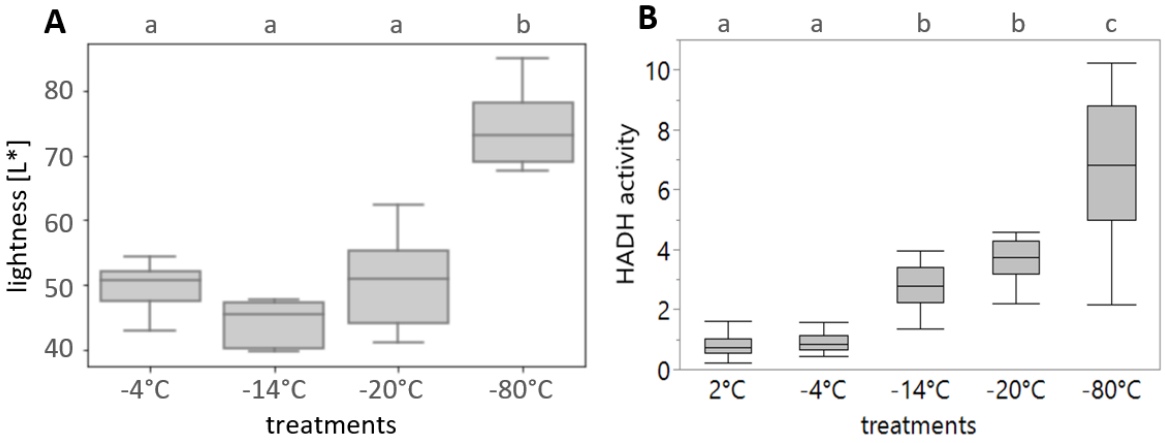

Fig. 1. Comparison of beef samples frozen at temperatures ranging from –4°C to –80°C. (A) Data for samples for which the frozen structure was preserved and assessed using the L* parameter. (B) HADH-activity for the same freeze treatments and for a refrigerated control. Letters indicate significant differences at P≤0.005 (Tukey's). Overall treatments were significant for both test assays (one–way ANOVA).

IV. CONCLUSION

Both methods allowed to separate very quick freezing at –80°C from freezing at higher temperatures. Similar to our previous study using bioimpedance–based testing [3], sub-zero frozen (–4°C) samples were not distinguishable from refrigerated only beef (2°C). This can have implications for understanding the extent of freeze damage at temperatures, where meat is only incompletely frozen.

ACKNOWLEDGEMENTS

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CARCASS TRAITS AND BEEF QUALITY OF FEEDLOT NELLORE BULLS DIVERGENT FOR STRIPLOIN TENDERNESS

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I. INTRODUCTION

Nellore cattle, the main beef breed raised in Brazil, may have greater calpastatin activity in the muscle, which results in less postmortem proteolysis and, consequently, tougher meat [1]. Tenderness, that is instrumentally measured through Warner-Bratzler shear force (WBSF) analysis, is one of the most important meat quality traits as it is highly associated with eating satisfaction [2]. However, because tenderness cannot be assessed at the moment of purchase, it is important to determine its association with other traits that could be more easily identified by consumers. Therefore, this study was conducted to characterize carcass traits and beef quality of feedlot Nellore cattle classified as high- or low-WBSF based on values measured in the *L. thoracis* (LT) muscle.

II. MATERIALS AND METHODS

This study was conducted with a dataset from another trial, in which 112 Nellore bulls with 20 months of age and 306 ± 38 kg of initial body weight (BW) were divided into 4 treatments (2 supplementation levels in the stocker phase vs. 2 adaptation periods in the feedlot finishing phase) and group-fed in 16 feedlot pens (7 bulls/pen). After 143 days on feed, final BW was taken, and real-time ultrasonic measurements were obtained from the LT muscle between the 12th and 13th ribs to determine ribeye area and backfat thickness. Then, 3 animals per pen were slaughtered in a commercial slaughterhouse, and carcass dressing was determined as the ratio of hot carcass weight (HCW) to final BW. Following a 24-hour chilling period, striploin samples were collected between the 12th and 13th ribs for later analysis of pH, instrumental color (with a Minolta BC10 colorimeter), thawing, cooking, and total losses. The WBSF was determined by shearing six 1.27-cm-diameter meat cylinders after samples were cooked to an internal temperature of 71°C and then cooled to 7°C. The original dataset with data from 48 carcasses was used to segregate cattle into 2 divergent WBSF groups: high (8.02 to 5.53 kgf) and low (4.28 to 2.40 kgf) WBSF. Animals from the 4 treatments of the original trial were equally distributed across both WBSF groups in the resulting dataset, and those treatments were considered as blocks in the statistical analysis to remove their effects. Therefore, the resulting dataset consisted of 2 treatments (high and low WBSF), 4 blocks (treatments from the original trial), 12 replicates per treatment, comprising 24 experimental units. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS, and significance was declared at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Although final BW was greater ($P = 0.05$) for high-WBSF bulls, HCW, carcass dressing, ribeye area, and backfat thickness did not differ between cattle naturally divergent for striploin tenderness (Table 1). Beef from low-WBSF bulls had higher pH ($P < 0.01$) than those from high-WBSF cattle, which has been associated with a more rapid tenderization of the meat due to the faster rates of degradation of muscle proteins by calpains at higher pH values (pH > 6.2) [3]. Moreover, slow rates of pH decline are

also associated with more tender beef when cold shortening is prevented [3], which likely occurred considering the 4.85 mm average backfat thickness for low-WBSF bulls. Higher beef pH is also related to greater water-holding capacity, thus explaining the lower values for thawing, cooking, and total losses, as well as for beef lightness (L*) in the meat of low-WBSF cattle [4].

Table 1. Carcass traits and beef quality of feedlot Nellore bulls divergent for striploin tenderness.

Item[1]	Treatment		SEM[2]	*P*-value
	High WBSF	Low WBSF		
WBSF, kgf	6.29	3.54	0.317	<0.01
Initial body weight, kg	309.50	303.50	7.073	0.49
Final body weight, kg	512.33	498.92	5.055	0.05
Hot carcass weight, kg	287.12	279.63	3.565	0.11
Carcass dressing, %	56.03	56.02	0.304	0.99
Ribeye area, cm^2	81.31	79.92	1.364	0.62
Backfat thickness, mm	5.60	4.85	0.213	0.08
pH	5.76	6.22	0.082	<0.01
Thawing losses, %	8.00	2.58	0.692	<0.01
Cooking losses, %	23.53	20.03	0.652	<0.01
Total losses, %	31.77	23.85	1.203	<0.01
L*	37.79	35.79	0.509	0.03
a*	12.31	8.52	0.645	<0.01
b*	6.52	5.01	0.366	0.04

[1] WBSF = Warner-Bratzler shear force; L* = lightness; a* = redness; b* = yellowness; [2] SEM = standard error of the mean.

IV. CONCLUSION

In summary, carcass traits were similar for Nellore bulls naturally divergent for striploin tenderness. Low-WBSF cattle had slightly darker beef with fewer thawing and cooking losses resulting from a greater pH.

ACKNOWLEDGEMENTS

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POSTMORTEM CHANGES IN WATER PROPERTIES OF WOODEN BREAST MEAT

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I. INTRODUCTION

The wooden breast (WB) condition is a myopathy occurring in the breast muscle of broilers that causes substantial losses to the industry due to product discards and downgrades. Breast meat exhibiting the WB myopathy is characterized by abnormal muscle hardness and rigidity. Compositionally, WB has greater moisture and connective tissue content and decreased protein content [1]. Previous research has shown that WB not only has undesirable texture attributes but also diminished water-holding capacity (WHC) [1]. The underlying factors controlling WHC in WB are not well understood. Changes in the mobility and distribution of water in muscle during the postmortem transformation of muscle to meat are thought to play important roles in determining meat WHC [2]. Thus, the objective of this study was to compare changes in water properties throughout the first 24 h postmortem in WB and normal breast meat using time domain nuclear magnetic resonance (TD-NMR).

II. MATERIALS AND METHODS

Broilers (56 day old, Cobb 500) were electrically stunned and bled. Breast muscles (*Pectoralis major*) were removed at <5 min postmortem from 15 normal carcasses and 15 carcasses exhibiting severe WB. Samples (10×10×2 cm) were removed from the cranial-ventral portion of each breast fillet. Samples were placed in plastic bags, chilled on ice, and stored at 4°C until 24 h postmortem. At 0, 0.5, 1, 2, 3, 5, 7, 12, and 24 h postmortem, transverse relaxation time (T2) was measured on samples using a time domain 1H NMR analyzer (LF 90II Proton-NMR, Bruker minispec). The T2 relaxation decays were analyzed using CONTIN software to estimate the time constants and relative proportions of water populations. At multiple time points throughout first 24 h postmortem samples were reweighed to calculate purge loss and pH measurements were recorded. Effects of WB condition, postmortem time, and their interaction were analyzed using a two-way ANOVA with repeated measures.

III. RESULTS AND DISCUSSION

Analysis of T2 data revealed 4 water populations in meat samples (Figure 1): T2b (4-5 ms, water bound to macromolecules), T21 (40-60 ms, intramyofibrillar water), T22a (140-210 ms, extramyofibrillar water with lower mobility), and T22b (350-550 ms, extramyofibrillar water with higher mobility). Water properties (mobility and distribution) changed most rapidly during the first 3-5 h postmortem and were then relatively steady until 24 h postmortem for both normal and WB meat (Figure 3). Similarly, purge loss increased most rapidly during the first 3 h postmortem, particularly for WB samples (Figure 2). With the progression of postmortem time, relative proportions of bound (P2b) and intramyofibrillar (P21) water increased ($P<0.05$) whereas extramyofibrillar (P22b) water decreased ($P<0.05$). The postmortem time point at which intramyofibrillar water changes (T21 and P21) leveled off was earlier in WB than normal samples. All 4 water populations exhibited greater ($P<0.05$) mobility in WB compared to normal meat. The relative proportions of bound and intramyofibrillar water were greater ($P<0.05$) in normal meat and the relative proportions of extramyofibrillar water were greater ($P<0.05$) in WB meat, similar to previous reports [3]. Maximum differences in water properties between WB and normal samples occurred ~3 h postmortem. The most dynamic changes in water distribution and mobility, purge loss, and pH (data not shown) occurred early postmortem suggesting that physical

changes associated with rigor mortis likely play a key role in controlling water distribution and WHC characteristics in breast meat.

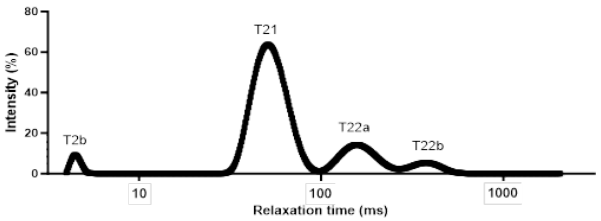

Figure 1. Continuous T2 relaxation spectra.

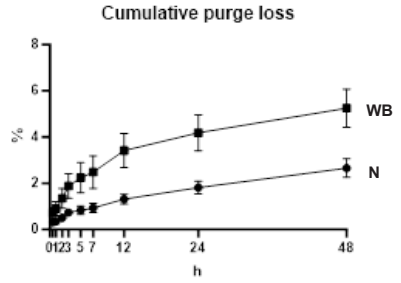

Figure 2. Cumulative purge loss of normal (N) and WB meat.

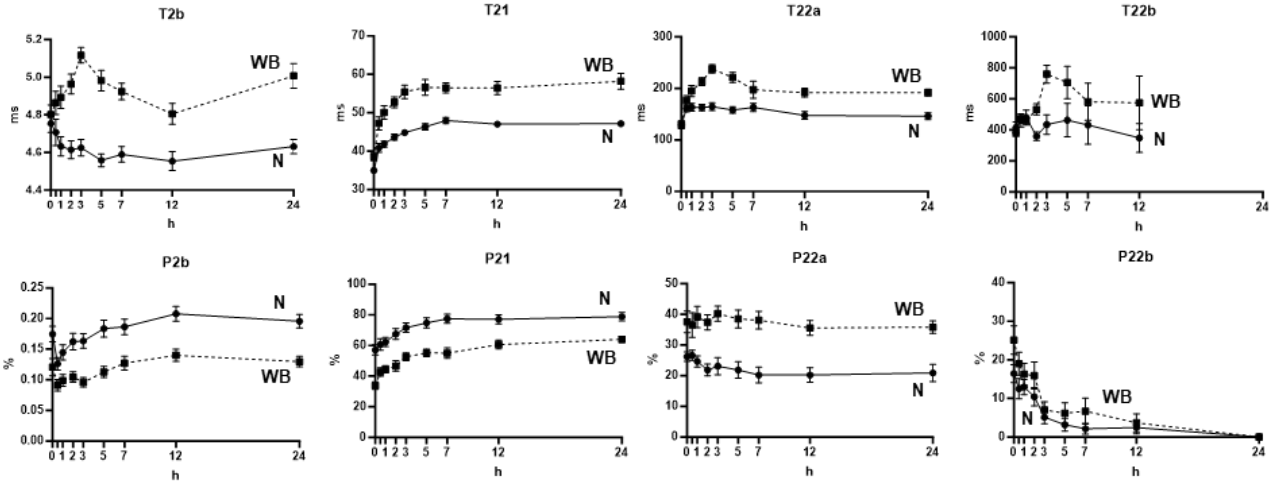

Figure 3. Water properties (mobility and relative proportions) of normal (N) and WB meat during 24 h postmortem: bound water (T2b, P2b), intramyofibrillar water (T21, P21), extramyofibrillar water with lower mobility (T22a, P22a), extramyofibrillar water with higher mobility (T22b, P22b).

IV. CONCLUSION

These findings demonstrated that broiler breast meat undergoes significant changes in water distribution within the tissue during the early postmortem phase (3-5 h). These postmortem water changes are more drastic in WB meat compared to non-WB meat and may be related to differences in the progression of rigor mortis development. Water in WB meat exhibited greater mobility than water in normal meat. These data provide further insight into the underlying mechanisms that control WHC and quality characteristics in broiler breast meat.

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The effect of a vascular rinse and chill on lamb carcase yield

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I. INTRODUCTION

The Rinse & Chill® Technology (RCT) uses an isotonic solution including water, dextrose, maltose and sodium phosphates as a method of vascular infusion to improve blood removal, lowering the internal carcase temperature more quickly and modifying carcase pH decline consequently, improving meat quality and safety [1]. While rinsing of lamb carcases has been previously shown to improve meat quality traits including tenderness with no difference on waterholding capacity of the loin [2], as yet no research has been conducted on the impact of RCT on carcase yield of lambs despite research in beef suggesting yield increases in the range of 5 – 6% are possible [1]. Therefore, the aim of this study was to assess the impact of RCT on lamb carcase yield.

II. MATERIALS AND METHODS

Over 5 days 1,450 carcases were randomly selected from a commercial lamb processing plant and included in the trial. Carcases were allocated via random block design to either RCT treated group or control group resulting in 664 treated carcases and 660 untreated controls as retained carcases were excluded from the trial (n = 126), resulting in a total of 1,324 observations. The RCT solution was infused based on 10% liveweight calculated using a pre-rinse weight for every individual carcase as per normal operating procedures. All other aspects of carcase processing remained as per standard industry practice.

Hot carcase weight (HCW) was recorded on entry to the chillers and GR Tissue Depth (110mm from the midline on the 12th rib) was also measured. At 24h post-mortem, 346 carcases were re-weighed to get a cold carcase weight (CCW). Linear models and ANOVA were used to calculate predicted means for HCW, CW and chiller shrink using kill as a fixed effect and pre- rinsed weight as a covariate using the ‘lem4’, ‘asreml’ and ’lmerTest’ packages in R Core Software [3]. Given pre-rinse weight tended to be higher for control carcases, analysis was completed by adjusting the models to a mean pre-rinsed weight.

III. RESULTS AND DISCUSSION

Overall, this trial demonstrated HCW was increased by ~3% when RCT was used. While there is a paucity of research in lamb, this is consistent with research on beef carcases which has shown early post mortem changes to the muscle as a result of RCT causes an increase in carcase yield of between 2 – 4% [4] This is demonstrated by models as increased HCW by 700g (± s.e 0.048), with a predicted mean HCW of 22.65 kg (95% CL 22.56 – 22.74 kg) and the mean weight of control carcases was 21.97 kg (95% CL 21.88 – 22.74 kg) as shown in Fig. 1. Explaining 82% of the variation in HCW, this model left a residual standard deviation of 1.2kg. While HCW was significantly different between days of measurement, no interaction was found between treatment and day.

Similarly, CCW increased by 700g per carcase with RCT treatment resulting in a mean CCW of 22.4kgs (95% CL 22.2 – 22.5kgs) compared to a mean CW of 21.7kg (95% CL 21.5 – 21.8). The fitted models explained 82% of the variation in CCW leaving a residual standard deviation of 1.1kgs. While the CCW also differed significantly between days, there was no interaction between day and treatment.

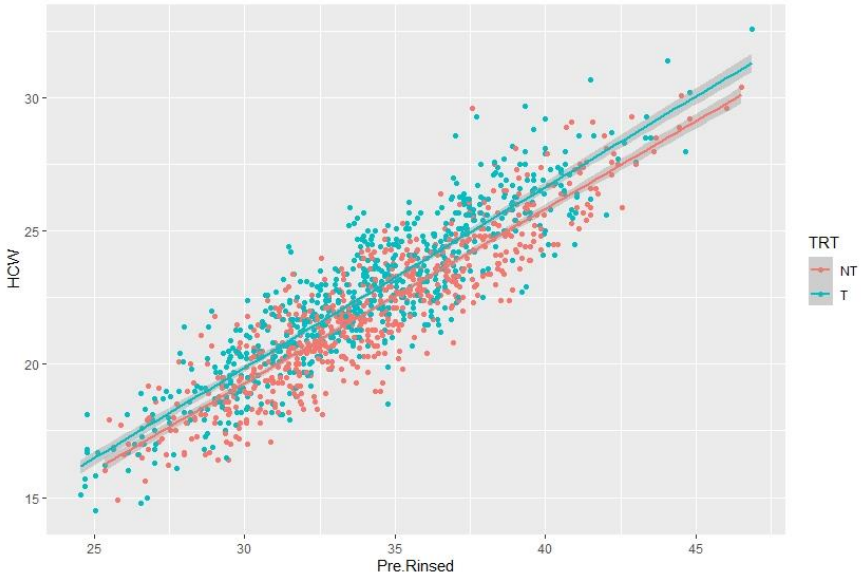

Figure 1. Pre-rinsed weight and hot carcase weight for RCT treated (T) and control (NT) carcases.

Although the reason behind the observed effect has not been described, Dikeman et al. [4] have hypothesised that increases in yield are the result of fluid retention which may have been achieved by altering the pH and temperature decline of carcases during the early post mortem period. It is postulated that metabolism of the solution by the muscle during the early post mortem period alters the myofilament lattice arrangement, denaturation of the myofilament heads and subsequently the extent of sarcomere shortening by altering the pH decline [5]. As a result, less water is pushed into the extracellular space and protein connections remain intact which prevents the shrinkage of the muscle during rigor being translated from the myofibrils to the whole muscle causing water loss. However, further work on the mechanisms is required.

IV. CONCLUSION

Overall, this study demonstrated treatment of lamb carcases with Rinse & Chill resulted in a significant increase of lamb yield, which is retained through chilling.

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Sous vide cooking improves the eating quality of spent buffalo (bubalus bubalis) meat

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I. INTRODUCTION

Tenderness is an important factor that influences overall consumer satisfaction, repeat buying decisions, and willingness to pay a premium price [1]. In South Asia, a large proportion of meat comes from spent animals, especially buffalo (Bubalus bubalis) [2]. Sous vide is a French cooking technique where meat in a vacuum-sealed bag is cooked under water at temperatures ranging from 50 ℃ to 85 ℃ for between 2 to 18 hours [3]. Improving the tenderness of meat from spent animals can impact consumer acceptance and provide an affordable animal protein source to cater for the growing protein demands in developing countries. This study assessed the effect of controlled time-temperature conditions in sous vide cooking on eating characteristics of buffalo meat.

II. MATERIALS AND METHODS

36 muscle samples (18 each of bicep femoris (BF) and semitendinosus (ST)) were obtained from randomly selected female spent buffalo carcasses (Bubalus Bubalis) aged > 60 months. The study comprised 6 treatments; a 3 × 2 factorial arrangement of treatments was applied according to a completely randomized block design with 3 specific cooking conditions, i.e., 55 ℃ for 8 h (55℃-8H), 65 ℃ for 5 h (65℃-5H), and 95 ℃ for 45 min (95℃-45M) and 2 muscle types, BF and ST with 3 batches as replications. BF and ST was cut to yield 2.5 cm thick steaks (250 g ± 10 g). Each steak was vacuum packed in impermeable bags and cooked in a water bath. At the cessation of cooking, the samples were cooled in an ice bath and kept overnight at 4-5 ℃. Tenderness was measured by WBSF texture analyzer, color by using Minolta colorimeter, cooking loss & cooking yield were recorded accordingly after removing cooked steaks from the vacuum bags. Samples were also analyzed for myofibrillar fragmentation index (MFI) and total collagen content (TCC). Samples were assessed for sensory analysis using 9- point hedonic scale after searing steaks on the hot plate. Data was analyzed using the mixed procedure of SAS (version 9.4). The Tukey-Kramer test was applied to compare means.

III. RESULTS AND DISCUSSION

The findings illustrated variation in mean WBSF values between cooking conditions ($p < 0.001$) for both muscles, and no difference in tenderness ($p = 0.19$) was observed among the muscles while analyzing muscle type as illustrated in table 1. However, findings of color were found to be significant in color parameters except C in meat type and L* among 65℃-5H and 95℃-45M. The mean total collagen content & MFI values showed differences in cooked meat between the cooking conditions and the muscles ($p < 0.001$) while cooking loss and cooking yield showed less significance ($p = 0.022$) illustrated in figure 1. Sensory evaluation results demonstrated that the sensory panelists liked meat from 55℃-8H cooking for all the sensory traits, followed by 65℃-5H and 95℃-45M.

Table 1: Effect of cooking temperature and muscle type on tenderness & color (a*, C, L*, b* & H) of cooked BF and ST steaks.

Cooking Conditions	*WBSF*	*a* *	C	L*	b*	H
55°C-8H	$28.6^{c} \pm 3.64$	$9.9^{c} \pm 0.37$	$14.8^{c} \pm 0.32$	$51.3^{a} \pm 0.29$	$10.8^{a} \pm 0.13$	$47.9^{a} \pm 0.89$
65°C-5H	$41.9^{b} \pm 3.64$	$11.2^{b} \pm 0.37$	$16.5^{b} \pm 0.32$	$44.7^{b} \pm 0.29$	$12.1^{b} \pm 0.13$	$46.9^{b} \pm 0.89$
95°C-45M	$55.9^{a} \pm 3.64$	$10.7^{a} \pm 0.37$	$15.4^{a} \pm 0.32$	$44.7^{b} \pm 0.29$	$10.9^{ac} \pm 0.13$	$45.5^{c} \pm 0.89$
P value	< 0.001	<.0001	< .0001	< .0001	< .0001	< .0001
Muscle type						
BF	42.5 ± 3.63	$10.9^{b} \pm 0.37$	15.5 ± 0.32	$45.3^{a} \pm 0.24$	$10.9^{a} \pm 0.12$	$44.7^{a} \pm 0.87$
ST	41.8 ± 3.63	$10.2^{a} \pm 0.37$	15.6 ± 0.32	$48.5^{b} \pm 0.24$	$11.7^{b} \pm 0.12$	$48.7^{b} \pm 0.87$
P value	0.19	<.0001	0.66	< .0001	< .0001	< .0001

*L** (Lightness), *a** (redness), *b** (Yellowness), C (Chroma), H (hue)
WBSF: Warner Bratzler's shear force; BF: *bicep femoris*; ST: *semitendinosus*

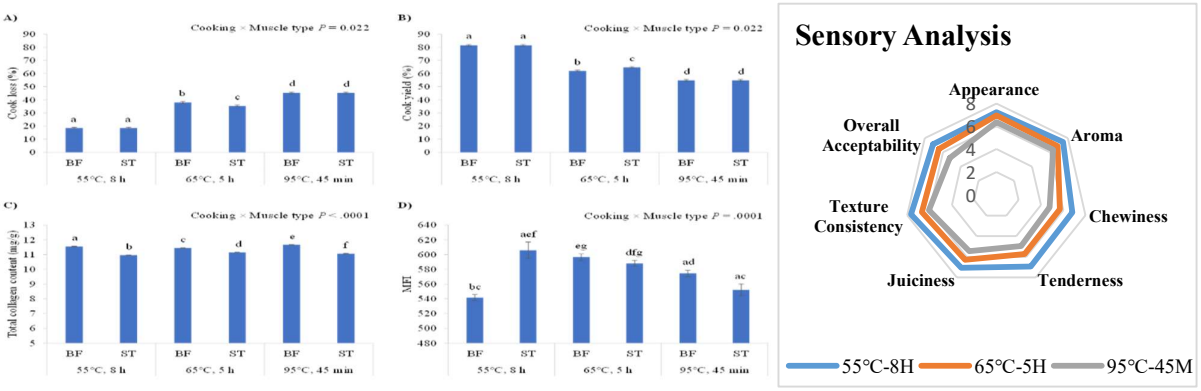

Figure 1. Effect of cooking conditions and muscle type on cooking loss, cooking yield, total collagen content & myofibrillar fragmentation index (MFI). While Figure 2 illustrate the effect of cooking temperature on sensory parameters

IV. CONCLUSION

This study has shown that cooking at constant low temperatures and time significantly improved the tenderness of the two muscles while preserving the organoleptic characteristics. low-value cuts can be utilized as steaks which can be available at a lower price. The findings of this study may also apply in the development of ready-to-cook and eat buffalo meat products.

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Text-mining assessment of hot boning applications in meat research and associated processing technologies: Analysis of current knowledge and research trends

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I. INTRODUCTION

Hot boning (HB) in meat processing is characterized by the removal of meat from the carcass shortly after slaughter (often within 90 minutes post-slaughter) [1]. One of the most significant advantages in HB is the potential reduction in energy expenditure. Since hot boning involves optimised cooling of edible meat only and not excess bone and fat, it has been estimated that hot boning could require 40-50% less chilling input, reduce chiller space by 50-55% and reduce labour by as much as 25% [2]. With the aim of exploring the different approaches and research trends related to HB in the literature, this paper investigates the multifaceted landscape of HB within meat quality research and processing, employing a text-mining approach. An analysis of the interconnected terms aims to offer a comprehensive look into the current research and processing trends related to HB in beef processing.

II. MATERIALS AND METHODS

A literature search was performed in the Scopus database up to April 2024, using the keywords “hot-boning” OR “hot boning” AND “meat” OR “quality” OR “trait” AND “beef” OR “bovine” or “cattle”. The search yielded 55 papers based on the titles, abstracts and keywords search. One paper dealing with horse meat was disregarded. The search data were then processed with VOSviewer software to identify the research trends and main clusters that characterize the keywords used. The analysis was further combined with in house review on HB on beef.

III. RESULTS AND DISCUSSION

The visualization clusters primarily highlight three thematic areas (Figure 1): meat quality and muscle biochemistry (red), safety (blue) and physicochemical properties of the meat (green). Central to these clusters is the concept of 'hot boning', which appears as a pivotal practice within the red cluster that closely links to 'meat,' 'tenderness,' and 'sarcomere length.' The red cluster suggests that a significant focus of hot boning research relates to its impact on meat quality, particularly tenderness - a critical attribute for consumer satisfaction. The data reflects that HB can influence meat tenderness by interacting with and influencing muscle physiology. For instance, when the temperature of muscles is low pre-rigor, extensive shortening of sarcomeres has been observed, and, as a consequence, the toughness increases in the process denoted cold shortening, as indicated by the terms 'sarcomere length' and 'tenderness' in close proximity. For cold shortening to occur, certain conditions must be met: the pH of the muscle has to be greater than 6.0, ATP should still be available and muscle temperature to be less than 10℃ [3]. To avoid such situations, different technologies can be applied such as electrical stimulation, slow chilling and hanging.

In the blue cluster, the terms *'Escherichia coli*,' 'food contamination,' and 'food microbiology' are prominent, revealing a strong linkage to meat safety concerns. In fact, HB could impact microbial growth due to the higher temperatures at which meat is processed. The proximity of these terms suggests that current research is investigating whether HB can either reduce or worsen the risk of microbial contamination. Considering the paramount importance of food safety within meat processing, understanding these dynamics is crucial for regulatory compliance and public health assurance.

The green cluster focuses on 'pH,' 'low temperature,' and 'hydrogen-ion concentration,' emphasizing the importance of physico-chemical dimensions during processing of meat and the final outcome in relation to meat quality. The manipulation of these properties during the HB process can affect the endogenous proteolytic systems through the rate and extent of pH decline, which are critical for several intrinsic quality traits. This signifies ongoing investigations into how HB influences these parameters that are crucial both quality and safety of beef.

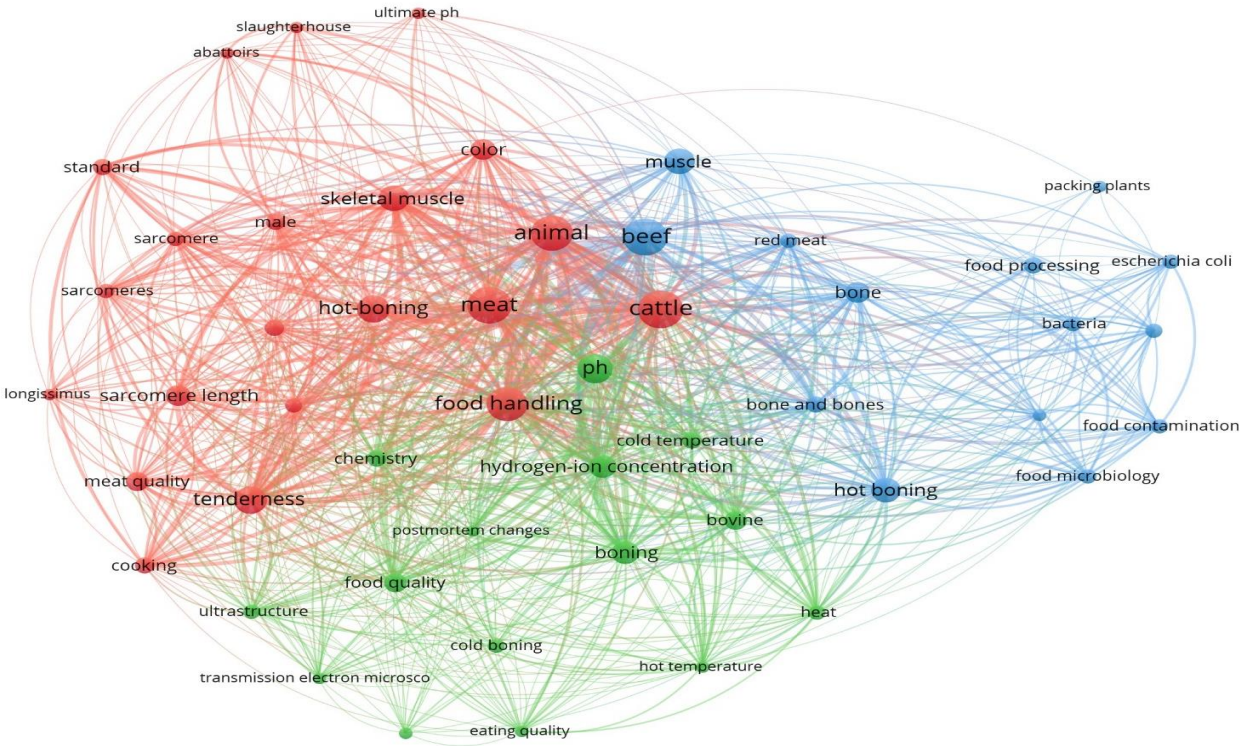

Figure 1. Network of most frequent terms based on their co-occurrence in the selected articles

IV. CONCLUSION

This study explored the applications of HB in meat research and processing. It offers a textualized understanding of how HB is situated \within the wider topics developed in meat science. While HB is linked to potential improvements in processing efficiency and meat quality, it also requires careful management of food safety. Future research should continue to explore these relationships, particularly through studies that assess the long-term impacts of HB on sustainability in the beef industry. Furthermore, the integration of consumer perception studies could synchronize processing innovations with market demands. This text-mining assessment not only underscores the multifaceted impacts of HB on the beef industry, but also directs attention towards integrated research methodologies that maximise quality, safety and sustainability - key factors for advancing a more resilient and consumer-responsive beef sector.

ACKNOWLEDGEMENTS

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EFFECTS OF SOYBEAN MEAL REPLACEMENT WITH UREA ON BEEF QUALITY AT DIFFERENT AGING TIMES

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I. INTRODUCTION

The substitution of true protein sources for non-protein nitrogen sources, such as urea (UR), has been employed to reduce feed costs in the livestock industry. Protein-rich feeds, such as soybean meal (SB), tend to be more expensive and they can represent a high cost during the cattle finishing phase [1]. The substitution of protein for non-protein sources could affect the characteristics of carcass and meat quality. In this context, the objective of this study was to evaluate the effect of partial or total replacement of SB by UR in the diets of F1 Red Angus x Nellore cattle on the qualitative parameters of beef aged for 0 and 14 days.

II. MATERIALS AND METHODS

Thirty F1 Red Angus x Nellore bulls, averaging 9 ± 1 months of age and 370 ± 6 kg, were randomly assigned to receive one of three experimental diets, containing different levels of replacement soybean with urea based on crude protein content, on dry matter basis: diet with 0% replacement (0% UR and 12% SB); diet with 54% replacement (1% UR and 5,6% SB); and diet with 100% replacement (1.9% UR and 0% SB). The experiment lasted 93 days (9 days of adaptation and 84 days of trial). The diet was offered ad libitum, with a forage-to-concentrate ratio 20:80, using corn silage as forage source. All diets were isoproteic, with approximately 13% crude protein/kg (DM basis), formulated for a gain of 1.3 kg/day, following the BR-CORTE [2] recommendations. At the end of the 93-day trial, the animals were harvested. After 24h of carcass chilling, a portion of the *longissimus lumborum* was extracted and divided into two properly identified and vacuum-packed portions: one was non-aged and immediately frozen, and the other aged at 2°C for 14 days. All samples, non-aged and aged, underwent freezing before being sent to the meat science laboratory, where they were cut into steaks for subsequent meat quality analyses. Meat color measurements (L^*, a^*, and b^*) were obtained using a Hunter MiniScan EZ colorimeter (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA). The wavelengths were used to determine the percentage of metmyoglobin (MMb), deoxymyoglobin (DMb), and oxymyoglobin (OMb), following AMSA equations [3]. Moreover, analyses of thawing and cooking losses, and Warner-Bratzler shear force (WBSF) were performed. Data were analyzed using SAS software (9.4; SAS Institute Inc., Cary, NC, USA). PROC GLM was employed to evaluate the effects of diet and aging and their interaction on meat quality parameters. Comparisons were performed using Tukey's test, considering a significance level of 5%.

III. RESULTS AND DISCUSSION

There was no interaction effect between dietary treatments and aging times (Table 1). The L^*, a^*, and b^* color parameters showed no difference among treatments or aging time. Also, there were no differences in the percentages MMb, DMb and OMb among the dietary treatments. However, aging times differences were observed in MMb (<0.001), DMb (<0.001), and OMb (P = 0.002), where 0-day showed lower MMb and DMb, and higher DMb compared to 14-days aged beef. Furthermore, there were no differences among the dietary treatments (P = 0.080) for WBSF. However, differences were observed among the different ageing periods (<0.001), with 14-day aged steaks presenting a better tenderness than 0-day. The lack of differences among the dietary treatments aligns with studies that found no significant impact of urea on carcass composition [1,4], indicating that urea in the diet does

not alter meat quality. The differences observed at different aging times were expected due to proteolysis and oxidation, which enhance tenderness and change the myoglobin chemical states [5].

Table 1 – Color parameters, water losses, and shear force values of beef evaluated for partial or total soybean meal replacement with urea at different aging times.

Treatments[1]	Diet			Aging		*P-value*		
	0%	54%	100%	0 d	14 d	Diet	Aging	D*A[2]
Parameters								
*L**	40.48±4.21	37.76±4.26	36.78±3.52	38.17±4.07	38.72±5.19	0.131	0.535	0.980
*a**	14.24±1.98	13.84±1.46	13.31±1.77	13.76±1.69	14.56±1.65	0.945	0.374	0.950
*b**	12.30±2.52	10.94±1.81	10.50±1.85	11.16±2.07	12.39±2.65	0.325	0.100	0.943
MMb (%)	15.37±2.06	16.95±1.81	16.68±1.76	16.41±1.93	22.88±7.08	0.327	<0.001	0.068
DMb (%)	34.08±8.67	35.47±6.64	39.82±7.27	36.64±7.44	20.25±12.20	0.313	<0.001	0.777
OMb (%)	50.55±10.12	47.58±7.62	43.50±8.14	46.96±8.53	56.87±9.78	0.581	0.002	0.873
Thawing Loss (%)	7.02±2.20	8.10±2.44	6.25±2.46	7.09±2.31	5.62±1.94	0.748	0.058	0.150
Cooking Loss (%)	11.82±2.88	12.75±3.96	11.92±2.32	12.19±3.16	10.25±3.41	0.913	0.066	0.290
WBSF (N)	40.40±8.77	54.72±21.41	42.38±7.90	45.83±15.11	30.69±8.73	0.080	<0.001	0.155

[1]0% = 0% replacement of soybean meal with urea; 54% = 54% replacement of soybean meal with urea; 100% = 100% replacement of soybean meal with urea; 0 d = 0 day of aging; 14 d = 14 days of aging. [2]D*A = interaction effect between dietary treatments and aging times.
Means followed by standard deviation.

IV. CONCLUSION

This study indicates that the partial or total replacement of soybean meal with urea maintains beef quality attributes. This finding supports the viability of using urea as a cost-effective alternative to soybean meal in beef cattle diets without compromising meat quality.

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Effect of intermediate ultimate pH beef over ageing time on *Longissimus lumborum* muscle proteome from grass-fed Nellore

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I. INTRODUCTION

The ultimate pH (pHu) is a crucial factor in beef quality, influenced by various factors such as animal diet, exercise, and pre-slaughter stress. These factors can lead to muscle glycogen depletion and result in abnormal pHu beef (> 5.80) [1]. In Brazil, the prevalence of beef classified as intermediate (5.8 to 6.19) or high pHu (≥ 6.2) accounts for about 40% of production, causing significant economic losses [2]. Hence, this study aims to evaluate the impact of intermediate pHu beef over ageing time on the *Longissimus lumborum* (LL) muscle proteome from grass-fed Nellore (*Bos indicus*) bulls, a topic of great importance in the field of animal science and meat quality.

II. MATERIALS AND METHODS

Three LL muscles from grass-fed Nellore bulls (30 - 35 months old) classified as intermediate pHu (pHu 5.8 to 6.19) were obtained from a commercial slaughterhouse. The muscles were divided into 2.5 cm thick steaks and assigned to vacuum-aging treatments: 1-d (72 h post-mortem) and 14-d vacuum ageing at 4°C. Samples from each ageing time were stored at −80 °C for proteomic analysis. Protein extraction was performed as described by Wiśniewski *et al*. [3]. LC-MS/MS analysis was performed on a Xevo G2-QTof mass spectrometer (Waters Corporation) coupled directly to the chromatographic system. Differentially abundant proteins (DAPs) over ageing time were defined through volcano plot analysis (fold change ≥ 1.2; $P \leq 0.05$). Enriched Gene Ontology terms and pathways were investigated using the open-source tool Metascape® ($P \leq 0.05$, minimum overlap of 3, and enrichment factor > 1.5).

III. RESULTS AND DISCUSSION

PCA discriminated beef at 1-d and 14-d ageing (Fig. 1A). Volcano plot analysis (Figure 1B) revealed 26 DAPs between ageing times comparison, of which 12 were overabundant at 1-d ageing, and 14 were overabundant at 14-d ageing. Enrichment analysis revealed ten enriched terms (Figure 1C), most of which were related to energy metabolism and muscle structure. Succinate-CoA ligase subunit alpha (SUCLG1), NADH dehydrogenase ubiquinone flavoprotein 2 (NDUFV2), ubiquinone biosynthesis monooxygenase COQ6 (COQ6) and NADH dehydrogenase ubiquinone one subunit C2 (NDUFC2) were abundant at 1-d ageing indicating increased oxidative metabolism, as also observed by Zhai et al. [4]. These results suggest increased oxidative stress in meat with intermediate pH, resulting in reduced proteolysis and degradation of structural proteins. The overabundance of candidate biomarkers for

tenderness, such as aldehyde dehydrogenase (ALDH2), myozenin-1 (MYOZ1), malate dehydrogenase, mitochondrial (MDH2), troponin T (TNNT1) and under abundance of heat shock protein HSPA5 observed in intermediate pHu beef at 14-d ageing compared to 1-d ageing are indicative of a delay in tenderisation, which partially explain the toughness of intermediate pHu beef, as reported by [1, 5].

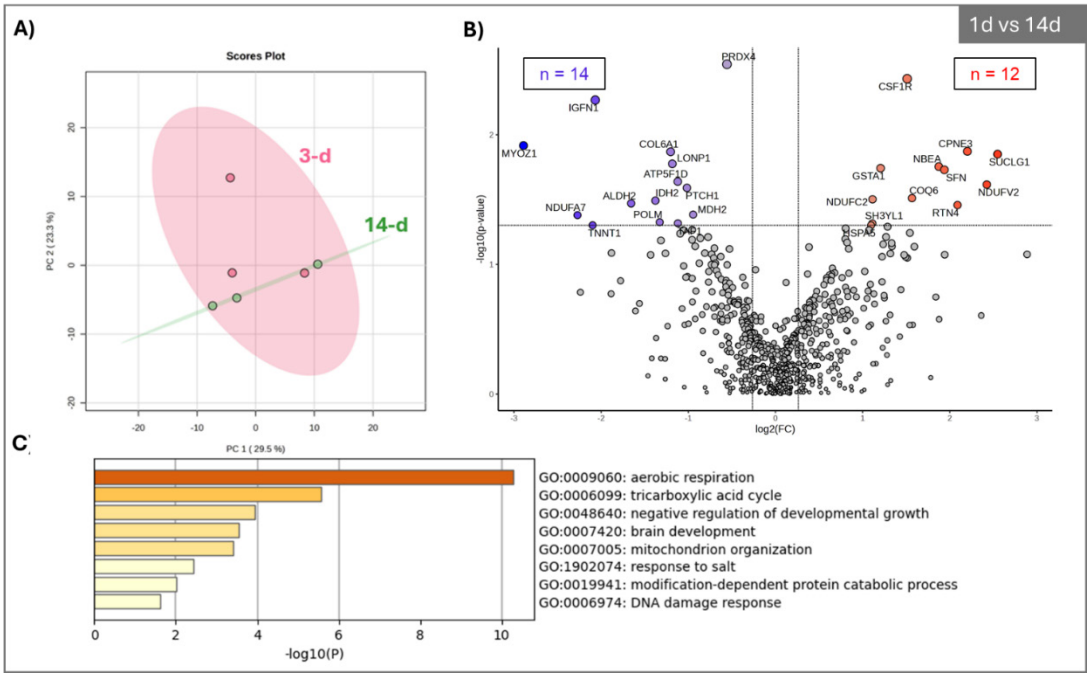

Figure 1. A) Principal Component Analysis (PCA); B) Volcano plot showing DAPs between 3 and 14 days of ageing; C) Bar chart of significantly enriched GO cluster terms according to *P*-values ($P \leq 0.05$).

IV. CONCLUSION

The main proteomic changes of intermediate pHu beef over aging time are related to energy metabolism and muscle structure, revealing that some proteins are essential for the main meat quality attributes, such as beef colour and tenderness.

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PRE-SLAUGHTER TRANSPORT STRESS ACCELERATED GLYCOLYSIS OF POSTMORTEM PORK: THE INITIAL REGULATORY ROLE OF S-NITROSYLATION ON INTRACELLULAR CALCIUM

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I. INTRODUCTION

Nitric oxide and its mediated protein S-nitrosylation have been reported to regulate the activity, localization, stability, and interaction of proteins and play an important role in determining meat quality [1]. In our previous study, pre-slaughter transport stress induced NO overproduction by promoting NO synthase activity, which thereby notably increased overall protein S-nitrosylation levels in postmortem pork [2]. Additionally, abnormal calcium release and uptake in the sarcoplasmic reticulum could induce intense muscle contraction, increased glycogenolysis, and faster pH drop. This work aimed to further explore the effect of pre-slaughter transport stress on intracellular calcium levels from the view of the regulatory role of S-nitrosylation on calcium transporters including ryanodine receptor 1 (RyR1) and sarcoplasmic reticulum calcium ATPase 1 (SERCA1) as well as thus the change in muscle glycolysis.

II. MATERIALS AND METHODS

2.1 Sample collection

A total of sixteen castrated crossed (Duroc × Landrace × Yorkshire) with an average weight of 110 kg were randomly selected and assigned to two treatments (n = 8): three-hour transport without resting (transport-induced stress group, TS group) and three-hour transport followed by three-hour resting (low-stress control group, CON group). The transportation and slaughter conditions have been stated in detail in our previous report [2]. At 30 min postmortem, the *longissimus thoracis* muscles between the 8th and 10th thoracic vertebrae were collected and stored at -80 °C for further analysis.

2.2 Glycogen content and lactic acid concentration

Glycogen content and lactic acid concentration of postmortem muscle were detected according to the methods of Ma et al [2].

2.3 Sarcoplasmic calcium concentration

The calcium concentration in the sarcoplasm was measured by employing inductively coupled plasma optical emission spectrometry referring to the procedures of Liao et al [3].

2.4 SERCA1 and RyR1 S-nitrosylation levels

The S-nitrosylated protein was enriched and purified using a pierce™ S-nitrosylation western blot kit (90105, Thermo Scientific, USA). The S-nitrosylation levels of the target protein were calculated by the ratio of enriched S-nitrosylated protein to total protein.

2.5 Statistical analysis

All data were presented as the mean ± standard deviation. The T-test was applied for significance analysis (Chicago, IL, USA). $P > 0.05$ means no statistical difference while $p < 0.5$ means significant differences.

III. RESULTS AND DISCUSSION

As Figure 1 (A and B) shows, compared with CON group, the glycogen content in TS group decreased by 28.9%, while the lactic acid concentration increased by 25.2% ($p < 0.05$), indicating an accelerated glycolysis metabolism in TS group muscle samples. The sarcoplasmic calcium concentration in the TS group was remarkably higher with an increase of 18.1% relative to CON group (Figure 1C, $p <$

0.05). An increased level of sarcoplasmic calcium in the early postmortem period was the primary contributor to the accelerated glycolysis in postmortem muscle [4]. Importantly, higher S-nitrosylation levels of RyR1 and SERCA1 in TS group were also found relative to CON group (Figure 1D-F, $p < 0.05$). The elevated S-nitrosylation levels of RyR1 and SERCA1 resulted from pre-slaughter transport stress could be responsible for the calcium overload in the cytoplasm through activation of RyR1 and inhibition of SERCA1.

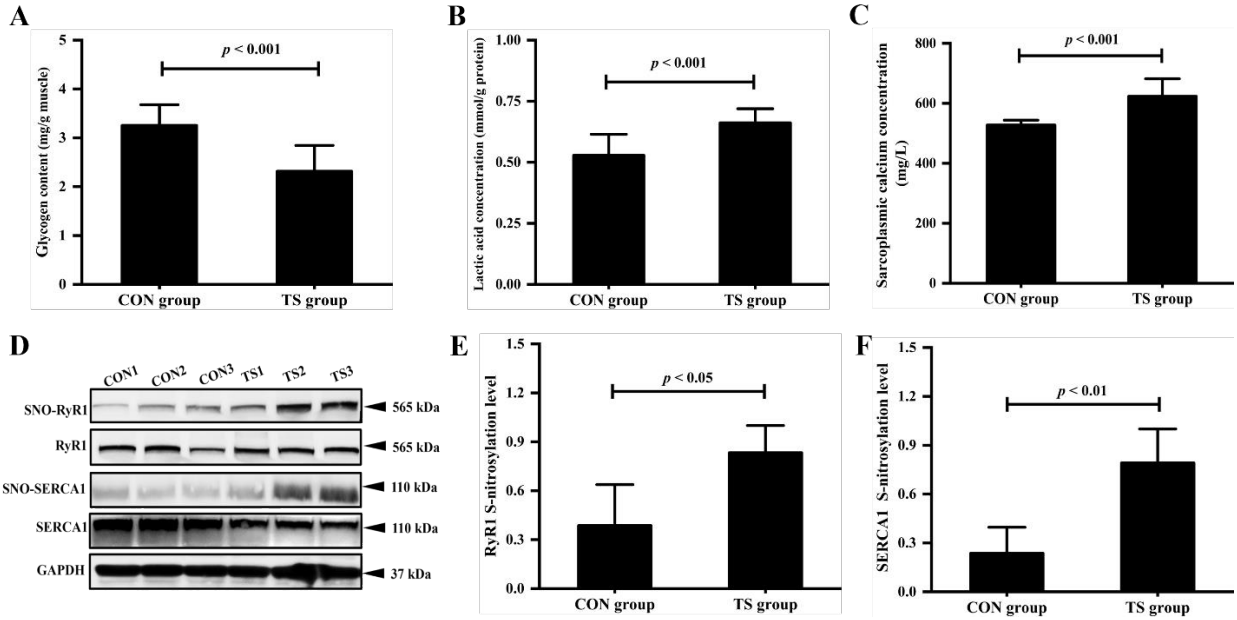

Figure 1. Differences in glycolytic metabolism (A and B), sarcoplasmic calcium levels (C), and calcium transporter S-nitrosylation levels (D-F) of postmortem *longissimus thoracis* muscle from pigs with different pre-slaughter stress levels. CON: control group; TS: transport-induced stress group.

IV. CONCLUSION

In summary, this study highlights that the increased S-nitrosylation levels of RyR1 and SERCA1 induced by pre-slaughter transport stress caused an increase in sarcoplasmic calcium, which further led to the acceleration of muscle glycolysis metabolism and thus the alteration of pork quality.

ACKNOWLEDGEMENTS

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ULTRASOUND-INDUCED MODIFICATIONS OF BEEF FLAVOR CHARACTERISTICS DURING POSTMORTEM AGING

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I. INTRODUCTION

The postmortem aging of beef leads to the accumulation of small molecules, such as free amino acids, small peptides, fatty acids, reducing sugars, and nucleotides, thereby enhancing the flavor characteristic of beef [1]. However, achieving the desired flavor and palatability of beef requires a prolonged aging period, which poses a significant expense for industrial production. Thus, it has significance to achieve an innovative way to shorten beef aging and remain or even improve beef flavor. Ultrasound, as a non-destructive, efficient, and environmentally friendly emerging technology, has been employed in the research of meat processing [2-3]. This work was designed first to explore the impact of ultrasound on beef flavor characteristics during postmortem aging.

II. MATERIALS AND METHODS

2.1 Sample collection

Four Simmental cattle with an average weight of 520 kg were selected and slaughtered. After carcasses were halved and placed in cold storage at 4 °C for 24 h, the *longissimus thoracis* muscle (pH ranging from 5.55 to 5.65) was extracted and collected. The samples were then promptly transported back to the laboratory within 6 h under controlled temperature conditions of 0-4 °C after vacuum packaging. At 48 h postmortem, each muscle was sliced into six 2.54 cm thick steaks and then individually vacuum-packed. Subsequently, all steaks were randomly allocated into two groups (n = 4): ultrasound (US) and control (CK) groups. The steaks in the US group were submerged in a water-filled (water bath at 0-4 °C) ultrasonic pot (Jining Tianhua Ultrasonic Electronic Instrument Co., Ltd., China) and sonicated for 40 min (20 min on each side, with a 5 min break at 20 min intervals), utilizing an ultrasonic power of 500 W at a frequency of 20 kHz. The steaks in the CK group underwent no sonication. After US treatment, all steaks were transferred to a cold room at 4 °C and aged for 0, 7, and 12 d for further analysis.

2.2 Gas chromatography-mass spectrometry (GC-MS) analysis

The method of Zou et al [2] was used for GC-MS analysis to identify volatile flavor compounds in various beef sample groups.

2.3 Statistical analysis

All data were analyzed by SPSS 20.0 software (Chicago, IL, USA), and the significant differences ($p < 0.05$) were determined by Duncan's multiple-range test in a one-way analysis of variance.

III. RESULTS AND DISCUSSION

A total of 69 volatile flavor compounds were identified in six groups, with 61 and 58 compounds being identified in US and CK groups, including 15 alcohols, 21 aldehydes, 4 ketones, 2 esters, 1 furan, 17 hydrocarbons, 4 acids, and 5 nitrogen-containing compounds (Figure 1). The relative content and type of volatile flavor compounds were significantly altered after US treatment ($p < 0.05$). Compared with the CK group, the relative content of the total volatile compound in the US group increased by 266.62%, 30.32%, and 18.02% at 0, 7, and 12 d of aging, respectively.

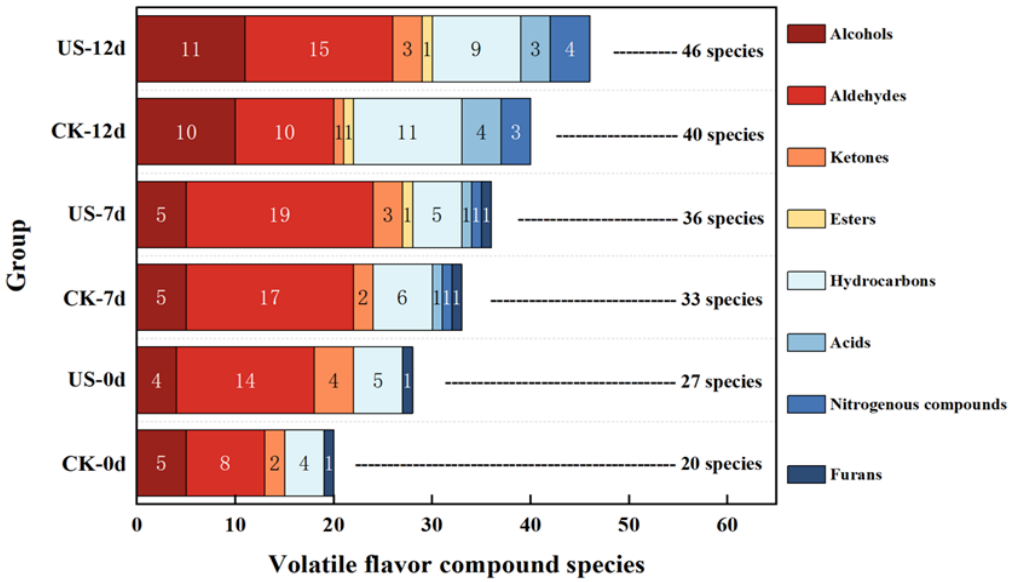

Figure 1. Volatile flavor compound of beef samples at different ultrasonic powers and postmortem aging time points. US: ultrasound group; CK, control group.

IV. CONCLUSION

In summary, this study shows that ultrasonic treatment increased the content and type of volatile compounds and improved the flavor characteristics of beef during postmortem aging. Thus, ultrasound technology can be regarded as an effective method for enhancing the beef flavor profile during postmortem aging.

ACKNOWLEDGEMENTS

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SESSION 6

Meat Safety

Tuesday 20 August 2024

PREDICTIVE MODELING OF MICROORGANISMS IN PORK USING DRIP METABOLITES AND ELASTIC NET REGRESSION

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I. INTRODUCTION

In pork, non-destructive prediction of the microbial populations and compositions is important for both economic and safety reasons. Metabolites in drip, which flow from meat due to environmental factors, have the potential to predict the condition of the meat [1]. When modeling to predict the condition of meat using metabolite data, it is crucial to prevent multicollinearity and overfitting due to variable interactions. Elastic Net regression addresses these issues by combining the variable selection capability of Lasso regression with the coefficient size determination of Ridge regression [2]. Therefore, our research aims to use Elastic Net regression to model the metabolites in pork drip for non-destructive predictions of microbial counts and composition, and to adjust it for predicting the specific microbial counts in meat.

II. MATERIALS AND METHODS

Longissimus thoracis muscles from both sides of three sows were purchased. They were cut to approximately 7 cm thickness and vacuum-packaged. A total of 30 loin cuts (5 experimental days × 3 animals × 2 sides) were stored at 4℃ for 27 days. The drip was collected using a 10mL syringe from vacuum-packaged samples. Total aerobic bacteria (TAB) count and microbial composition by 16S rRNA were measured in meat. Metabolites in drip were measured by nuclear magnetic resonance. The 16S rRNA was analyzed at 3 points (4, 13, 20 days). The obtained metabolite and microbial composition data were augmented using the Noise method with Python (n=500, N=1,500) [3]. The model was trained using augmented data, and the original data were used to validate the model. Normalization was performed with Python [2]. Elastic net regression was carried out in R [2].

III. RESULTS AND DISCUSSION

We examined the Net Elastic regression technique as a computational approach to predict metagenomic sequencing outcomes based on metabolite characteristics, integrating biological insights through taxonomic or functional profiles. The derived taxonomic profiles are calculated as the weighted totals of the relative abundances of metabolite features, using the regression coefficients from the Elastic Net model as weights in the prediction algorithm. The microbial profile and metabolites of pork used in the model (Figure 1). During the storage period, 17 types of microbial genes and 64 metabolites were identified in pork, with the TAB count increasing from 3.73 to 7.62 log CFU/g.

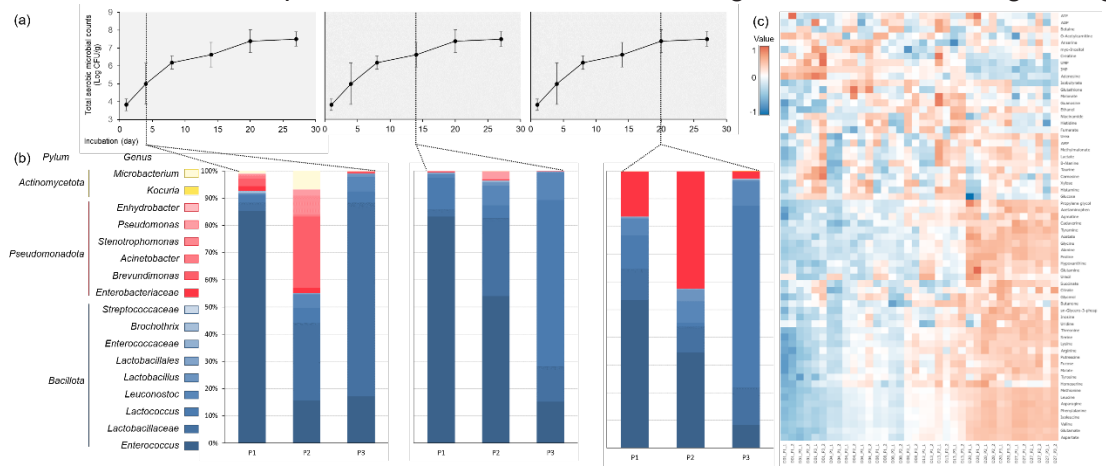

Figure 1. Changes in total bacterial count (a), microbial composition (b), and metabolites (c) during storage of pork.

The Percent model applied Elastic Net regression using metabolites from pork drip and the microbial composition. The Count model incorporated pork drip metabolites with data obtained by multiplying the microbial composition by the TAB count (Figure 2a). Spearman values increased for 12 types of microorganisms when the TAB count was applied to predict microbial composition (Count model). After applying unaugmented data to the models, the differences between predicted and actual values were confirmed through multidimensional scaling (MDS; Figure 2b). For comparison between the percent model and the count model, the scale of MDS 1 and MDS 2 was applied identically to the axes of the percent model. Compared to the percent model, the predicted MDS1 value for microorganisms is reduced in the count model, which suggests that the TAB count positively influences the model.

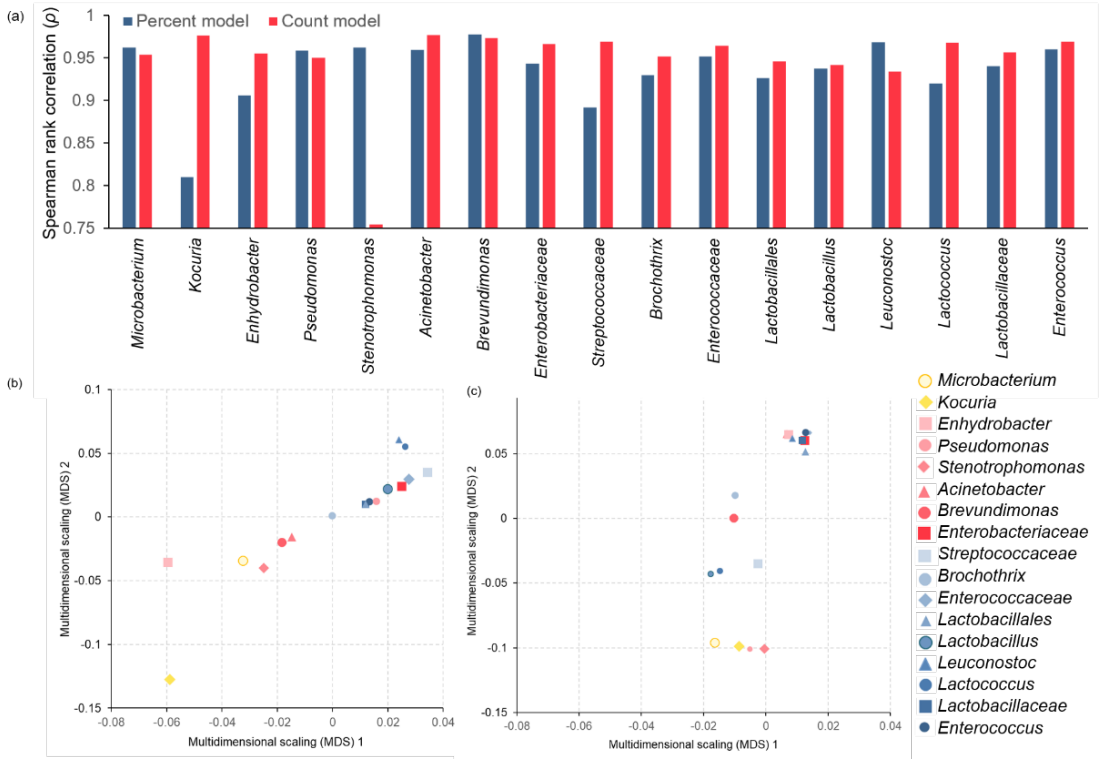

Figure 2. Assessment of microbial predictions by Elastic Net regression models. Spearman rank correlation (a), multidimensional scaling (MSD) of percent model (b) and count model (c).

IV. CONCLUSION

The Elastic Net regression model was constructed using the metabolites from pork drip and the microbial composition of pork. The counting model was able to predict the microbial characteristics using the metabolites of pork drip. Consequently, the metabolites in drip can be utilized to predict the microbial status of pork without destructive analysis. However, the concentration of metabolites and microbial composition in pork and drip can be influenced by environmental factors, so additional batch studies and considerations of collection conditions like packaging and storage are necessary.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant-funded by the Korea government (MIST; 2022R1A6A3A01085938).

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Staphylococcal Enterotoxin A (SEA) Reteined in Cells and Its Degradation in a Model Gastric Juice

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I. INTRODUCTION

Staphylococcus aureus contaminates foods such as meat products, dairy products, and other processed foods (Tsutsuura and Murata, 2017). *S. aureus* has various virulence factors such as toxins, adhesins, superantigens, and proteases. Staphylococcal enterotoxin A (SEA) which causes staphylococcal food poisoning in humans is one of the bacterial exotoxins produced by the bacteria. The potency of *S. aureus* to produce SEA differs among strains, and the SEA produced in food is very stable (Tsutsuura et al., 2013). In this study, we examined the change in the amount of SEA retained in the bacteria after SEA production in detail and whether digestive enzymes degrade it in gastric juice.

II. MATERIALS AND METHODS

After pre-cultivations, each BHI broth was inoculated with SEA-producing *S. aureus* suspension containing each of the eleven strains at 10^2 and 10^6 CFU/mL and incubated with shaking at 10, 15, and 37ºC (Tsutsuura and Murata, 2017). The culture medium was centrifuged at 10,000 g for 15 min at 4°C to collect cells. The precipitate was washed with PBS, resuspended in SDS-PAGE sample buffer, and then boiled for 5 min. After centrifugation, the supernatant was used as the bacterial internal extract. This and the culture supernatant were subjected to western blot analysis. Simulated gastric juice (pH 1.5) was made following Bornhorst and Singh (2013). The gastric juice was added to the culture supernatant or precipitate obtained by centrifuging the culture medium and then incubated with shaking at 37°C for 0.5-1 hr. After centrifugation, 1.5 M Tris-HCl was added to the supernatant and then subjected to western blot analysis. The staphylococcal counts were determined by colony-counting on mannitol salt agar plates. SEA was determined with western blot analysis. The detection limit was about 0.5 ng/mL.

III. RESULTS AND DISCUSSION

Figure 1 shows the growth of *S. aureus* (C-77-L22) and its SEA production inside and outside the cells when the bacteria were inoculated with inoculum sizes of 10^2 and 10^6 CFU/mL and incubated at 37°C. With both inoculum sizes, SEA was not detected inside the bacterial cells at the early stage of growth, but only detected outside the cells. It was suggested that the SEA produced in the early exponential phase was not retained inside the cells but was rapidly released outside the cells. The time required for SEA production was longer at 10°C and 15°C than at 37°C but the rate of SEA production inside and outside the bacteria was similar to that at 37°C. From these results, the SEA in the bacteria may retain a little or temporarily, or may be eluted from inside to outside due to the death of bacteria.

Figure 2 shows the appearance of SEA bands on SDS-PAGE before and after gastric juice treatment which were detected by western blot. The SEA bands disappeared after 0.5-1 hr of incubation at 37°C with artificial gastric juice added to purified SEA or culture medium containing SEA.

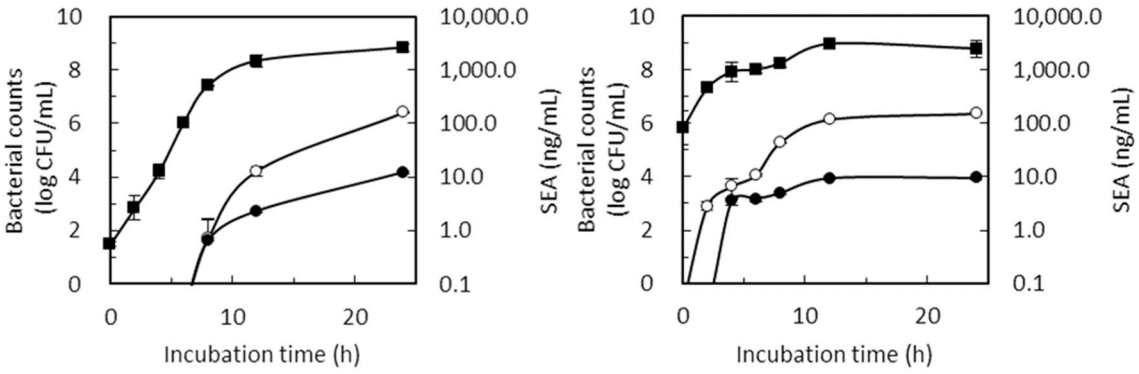

Figure 1. Growth (■) and intracellular and extracellular SEA (● and ○) of *S. aureus* with inoculum sizes of 10^2 CFU/mL (Left) and 10^6 CFU/mL (Right) in BHI broth at an incubation temperature of 37°C (n = 3).

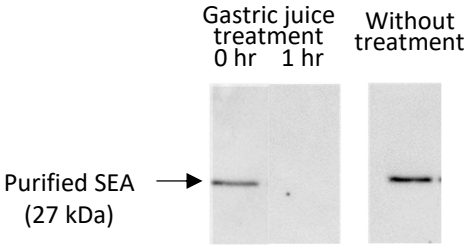

Figure 2. SEA bands before and after gastric juice treatment on western blot analysis

IV. CONCLUSION

In this study, the amounts of extracellular and intracellular SEA of *S. aureus* were quantified. The results showed that the intracellular SEA was as much or less than the extracellular SEA, suggesting that SEA was temporarily retained in the bacteria, and was quickly released after production. We also clearly showed that SEA was digested in a model gastric juice. Further study will be needed to evaluate SEA digestion under various conditions imitating digestion in the human stomach.

ACKNOWLEDGEMENTS

This study was supported by a Grant-in-Aid for Scientific Research (no. 23K2031 and 19K14007) from the Japan Society for the Promotion of Science and by Urakami Foundation for Food and Food Culture Promotion (no. R02519).

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STUDY OF THE EFFECT OF SODIUM LACTATE ADDITION AND FERMENTATION TEMPERATURE ON *LISTERIA MONOCYTOGENES* INACTIVATION DURING SALAMI PRODUCTION

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I. INTRODUCTION

Listeria monocytogenes is a bacterium that may cause listeriosis. *L. monocytogenes* is commonly found in meat and meat products such as salami. In salami, lactic acid fermentation is used as a preservation method through pH reduction and competitive exclusion in a medium containing between 2 - 3 % of sodium chloride. The fermentation and drying conditions will determine *L. monocytogenes* growth, conditioning the decrease in pH and water activity (aw) until reaching the restrictive values (pH ≤ 4.4 or aw ≤ 0.92 or pH ≤ 5.0 and aw ≤ 0.94) for *Listeria* growth. Sodium lactate (SL), used as pH regulator for sensorial purpose, has been reported to retard the growth of many microorganisms, including *L. monocytogenes* [1], and LAB [2]. The aim of the present work was to study the effect of SL and fermentation temperature (Tferm) on *L. monocytogenes* inactivation.

II. MATERIALS AND METHODS

A challenge study was conducted using pilot-scale salami production inoculated with *Listeria innocua* as a surrogate microorganism for *L. monocytogenes* [3]. Salami samples were prepared in 25 kg batches within a pilot plant. The meat and fat mixture (75:25), was ground to a fine grain using a cutter (LASKA KR 60-2 MV, Linz, Austria). Subsequently, the minced mixture was transferred to the blender (LASKA T WU5 Vac/P, Linz, Austria) and 150 ppm sodium nitrite, 2.6 % sodium chloride, 0.1 % ascorbic acid, 0.40 % polyphosphate, 0.03 % peppercorns, 0.10 % ground nutmeg, 0.30 % coriander, 0.10 % dehydrated garlic, 1% dextrose and starter culture (FLC, Chr. Hansen) were added. Salami were prepared with and without SL (0 and 2 %). This mixture was homogenized and inoculated with 10^7 CFU/g of *L. innocua* ATCC 33090. After mixing, salami were stuffed in an artificial casing 9.15 cm caliber using a HANDTMANN VF 12 – 100 sausage stuffer (Servo, Poland). Salami were fermented at 27 or 30 ºC in an ALFA LAVAL LR-6 chamber (Surrey, United Kingdom) at relative humidity (RH) of 90 ± 5 % for 2 days, and then, dried for 26 days in an ALFA LAVAL kkt 21021 chamber (Surrey, United Kingdom) at 17 ± 0.4 ºC at RH of 78 ± 5 %. *L. innocua* counts and pH of salami were determined at 0, 1, 2, 7, 14, 21 and 28 days. *L. innocua* counts were obtained by plating appropriate dilutions on Palcam Agar Base (Oxoid Ltd., Hampshire, UK) and incubating at 37 ºC for 48 hours. Water activity was measured at 0, 2, 7, 14, 21, and 28 days. *L. innocua* counts for each test condition over time were adjusted with Baranyi & Roberts equation using the software DMFit. *L. innocua* reduction in $\log_{10}$ CFU/g between the counts of *L. innocua* at the beginning of the fermentation and the counts reached at the end of the drying stage, estimated by Baranyi & Roberts equation, was calculated for each test condition.

III. RESULTS AND DISCUSSION

Listeria reduction was significantly greater at Tferm of 30 ºC than at 27 ºC (Figure 1). The addition of 2 % SL to salami interfered with *Listeria* reduction and pH drop (Figure 1 and 2) at both fermentation temperatures. The impact of SL addition on pH drop was greater at Tferm of 27 ºC than at 30 ºC. For all conditions, after 7 days the salami reached the restrictive aw (< 0.92) for *Listeria* growth and no effect of SL nor Tferm was observed.

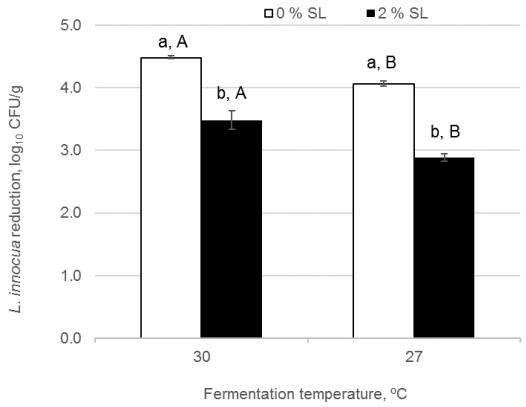

Figure 1. *L. innocua* reduction in presence or absence of sodium lactate (SL) at fermentation temperature 30 ºC and 27 ºC. The mean of two samples ± standard deviation of the values is presented. Different uppercase letters indicate significant differences (LSD, $p < 0.05$) between the means at different fermentation temperature for the same SL condition. Different small letters indicate significant differences (LSD, $p < 0.05$) between samples with 0 and 2 % SL for each fermentation temperature.

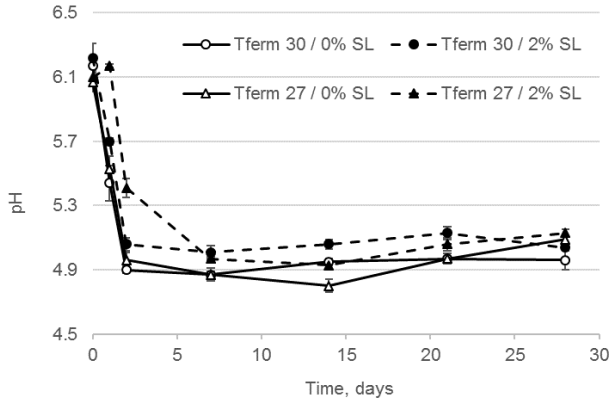

Figure 2. Evolution of pH at different fermentation temperature (Tferm) and in presence/absence of sodium lactate (SL). Each point represents the mean value of two samples ± standard deviation.

IV. CONCLUSION

The use of 2 % sodium lactate is not recommended since it compromises product safety by not allowing a reduction of *Listeria* greater than 3 $\log_{10}$ CFU/g as recommended by USDA-FSIS [4]. The addition of lower concentrations of sodium lactate should be studied.

ACKNOWLEDGEMENTS

The authors wish to thank ANII for financially supporting the Project FMV_3_2018_1_148907.

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WHAT IS THE REAL IMPACT OF *HAFNIA ALVEI* DURING COLD-STORAGE OF VACUUM-PACKED BEEF?

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I. INTRODUCTION

The microbiota composition of vacuum-packed (VP) beef product is diversified and includes desirable and also undesirable bacteria. Among these bacteria, it has been shown in previous works that the psychrotrophic bacterium *Hafnia alvei* was often detected in high abundance in the microbiota of vacuum-packed beef during cold-storage, but no direct impact on the product shelf life was identified [1]. Other studies recognized *H. alvei* as potential spoilage agents in VP beef but with no demonstration of their spoiling activity [2]. *H. alvei* belonged to the former *Enterobacteriaceae* family (hygiene indicator) until that the *Enterobacteriales* order was refunded into seven families, of which the *Hafniaceae* fam. nov., a family in it's own right [3]. Thus, the objective of the study was to investigate the behaviour and the impact in meat spoilage of *Hafnia alvei* strains during cold-storage, growing in VP beef.

II. MATERIALS AND METHODS

To explore the impact of *H. alvei* in the meat shelf life, challenge tests and sensory analysis were performed in parallel. Challenge tests were realized using eye round (*M. semitendinosus*) muscles collected from three suppliers in the cutting workshop. These tests were realized on two types of eye round meat: a sterilized meat (cauterized matrix : CM) and a natural meat (uncauterized matrix : UM). Eye round muscles were cut in sterile conditions into slices and were then inoculated by using strains from the UCMA collection (University of Caen Normandy), either *Hafnia alvei* UCMA 14205 (S1) or *H. alvei* UCMA 17635 (S2) strains. Uninoculated CM and UM meat was used as control. Meat cuts were vacuum-packed and kept following two scenarios, a professional (industrial) circuit (named A) with a temperature of 4°C for 40 days and a consumer's circuit (named B) with a temperature of 4°C for 14 days and 8°C for 7 days. Experiments were accomplished in triplicate. At each sampling time, microbial enumeration (presumed *Enterobacteriaceae sensu lato* in NF ISO 21528-2 then aerobic mesophilic flora in NF ISO 4833-1) and sensory analysis tests according to a grid for the conservation status of beef (odour, colour, exudate, blowing and overall impression), were realized. The overview of the experiment is shown in Figure 1.

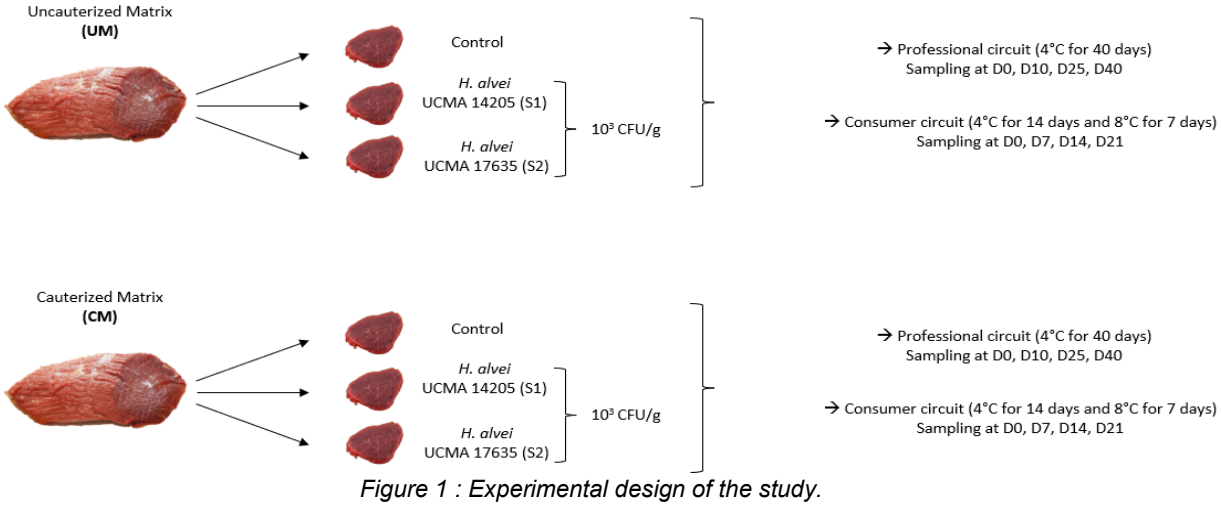

Figure 1 : Experimental design of the study.

To observe *H. alvei* behaviour within a natural microbiota, 16S metagenetic analyses were conducted on UM samples at the end of the conservation (D25 and D40 for circuit A and D14 and D25 for circuit B). Total DNA was extracted and the V3-V4 region of the 16S rDNA was amplified and sequenced on Illumina MiSeq. Bioinformatic and statistical treatment of data were performed in Galaxy solution [4].

III. RESULTS AND DISCUSSION

For the CM, the level of presumed *Enterobacteriaceae* increased over the conservation time both in the circuits A and B, as counts were around 10^6-10^7 CFU.g^{-1} at D40 and at D21 for the conditions inoculated with S1 and S2 strains, while counts were below the quantification threshold (10^1 CFU.g^{-1}) in the control condition. The results were globally the same for aerobic mesophilic flora, showing that the cauterization was effective at D0 (control condition) and that *H. alvei* was also enumerable with this method. Concerning the sensory analyses for the CM, no product was classified as unsatisfactory, some products were acceptable (10/72) and the majority were satisfactory (62/72) during the entire experiment. For the UM, the counts of presumed *Enterobacteriaceae* also increased along the conservation time but were slightly lower than in CM, as 10^5-10^6 CFU.g^{-1} counts were enumerated at the end of the conservation for the conditions inoculated with S1 and S2 strains. As expected in a natural matrix, aerobic mesophilic flora was detected at D0 around 10^1 CFU.g^{-1} and reached $10^{5\text{-}6}$ CFU.g^{-1} at the end of the conservation in both circuits. As for CM, no product was classified as unsatisfactory for the sensory analyses, some products were acceptable (9/72) and the majority were satisfactory (63/72) during the entire experiment. The 16S metagenetic analysis showed that Lactic Acid Bacteria dominated in the majority of the samples analyzed, with *Dellaglioa* and *Lactococcus* genera found in high proportions (often > 50% of abundance). The *Hafnia* genus was detected in proportions varying from 6% to 30%, principally at the end of the conservation process. Other genera, such as *Photobacterium*, *Carnobacterium* or *Latilactobacillus* were also detected. The statistical analyses of metagenetic also revealed that the origin of the meat impacts the evolution of bacterial composition.

IV. CONCLUSION

This study showed that *H. alvei* seems probably not correlated with beef spoilage. This work also showed the necessity to develop other methods to understand spoilage scenarios in meat, in order to have microbial indicators more informative of the state of conservation of the products. Globally, these issues still need to continue to be developed in order to reduce food waste and to ensure healthy fresh meat products to the consumer.

ACKNOWLEDGEMENTS

This work was funded by Interbev and the authors thank the colleagues from Institut de l'Elevage for helpful discussions, the professionals who provided the meat muscles and the INRAE MIGALE bioinformatics facility for providing computing and storage resources.

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Multi-layer films based on furcellaran containing active ingredients (curcumin extract, MMT, AgNPs and capsain) for storing pork loins

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I. INTRODUCTION

Food packaging plays an important role in ensuring food safety and protection, which in turn may lead to a reduction in food waste. Biopolymers such as proteins and polysaccharides are becoming interesting building blocks for packaging materials due to their environmental benefits. Furcellaran is a negatively charged polysaccharide obtained from the red alga *Furcellaria lumbricalis* and due to its gel-forming properties, it is an interesting candidate as a building compound for packaging materials. Moreover, it is non-toxic and biocompatible with other active ingredients, therefore it does not block their active action. Active packaging is gaining popularity because, due to the presence of an active additive, it can extend the shelf life of a food product. The aim of the work was to use two types of films previously characterized (1,2) as packaging materials for a meat product.

II. MATERIALS AND METHODS

2.1. Materials

All materials and reagents used in the experiment are presented (1,2). The procedure for obtaining gelatin hydrolysate was presented earlier (3). Fresh pork loin slices were obtained from local retail chain located in Kraków, Poland.

2.2. Methods

2.2.1. Preparation of active films

FILM A- The procedure for obtaining the FILM A is presented in the publication (1), and also schematically in Figure 1. **FILM B-**The methodology for obtaining FILM B was presented earlier (2) and in **Figure 1**.

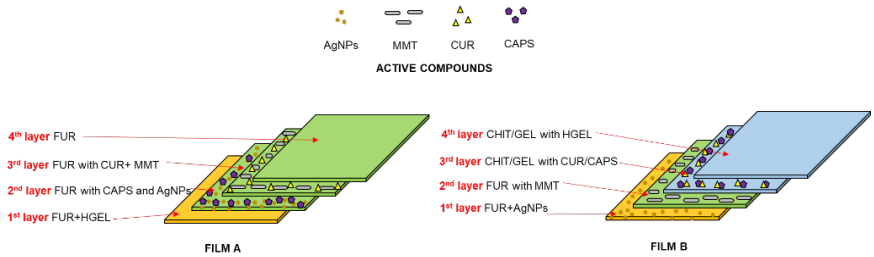

Figure 1. Scheme of obtaining two types of films used in an experimental study on a food product.

2.2.2. Evaluating the film influence on the quality of pork loins during cold storage

The pork loins slices were divided into 4 groups: wrapped in FILM A, FILM B and two controls: slices wrapped in LDPE film and not wrapped in any film. Samples were hermetically packed using tray sealer machine and stored at 4°C for 15 days. The samples were analyzed on days 0,4,7,11 and 15 for their microbiological contamination (total viable counts (TVC) and yeast and moulds (YM)), fat oxidation (TBARS) and sensory scores (color, aroma, texture and overall acceptability) according to (4). The experiment was carried out using three independent replications. Analysis included the two-way ANOVA with Tukey Post-hoc test with significance of difference established for $p<0.05$.

III. RESULTS AND DISCUSSION

The use of films caused the drying of the pork loin surface. This in turn resulted in inhibition of microbial growth, both TVC and YM. The TVC gradually increased from initial 1.8±0.1 log cfu/g on day 0 to 7.7±0.4 and 8.4±0.3 log cfu/g for control and LDPE control respectively on day 15. At the same time samples wrapped in films contained 3.5-5.1 log cfu/g of TVC, with the highest efficiency observed for FILM B. Similar trend was observed for YM, with control increasing from 0.8±1.4 to 6.8-7.8 log cfu/g vs 3.6-4.7 log cfu/g observed in samples wrapped in studied films (**Table 1**).

Table 1. Changes in (A) TVC (B) YM (C) TBARS.

	Days				
	0	**4**	**7**	**11**	**15**
TVC					
Control	1.77^{a}	1.94^{ab}	3.53^{bce}	5.77^{ef}	7.68^{g}
LDPE	1.77^{a}	2.22^{ab}	4.76^{def}	5.88^{f}	8.41^{g}
FILM A	1.77^{a}	2.28^{ab}	2.63^{abc}	4.13^{cde}	5.13^{def}
FILM B	1.77^{a}	1.87^{ab}	1.85^{a}	2.44^{ab}	3.53^{bce}
YM					
Control	0.81^{ab}	0.55^{a}	2.42^{abcde}	4.99^{ef}	6.81^{fg}
LDPE	0.81^{ab}	0.89^{ab}	3.52^{bcde}	4.83^{ef}	7.79^{g}
FILM A	0.81^{ab}	1.42^{abcd}	1.60^{abcde}	4.00^{de}	4.67^{ef}
FILM B	0.81^{ab}	1.11^{abc}	0.00^{a}	2.65^{abcde}	3.65^{cde}
TBARS					
Control	0.19^{ab}	0.17^{a}	0.21^{abc}	0.19^{a}	0.33^{abcd}
LDPE	0.19^{ab}	0.17^{a}	0.17^{a}	0.33^{abcd}	0.20^{ab}
FILM A	0.19^{ab}	0.33^{abcd}	0.39^{abcd}	0.46^{de}	0.42^{bcd}
FILM B	0.19^{ab}	0.28^{abcd}	0.25^{abcd}	0.43^{cd}	0.68^{bcd}

a,b,c -.Different lettering in the same rows indicate significant differences ($P < 0.05$)

On the other hand as mentioned, the films caused drying of the meat surface dry matter increased from 29.0 on day 0 to 33.3-40.1% after 15 days of storage), which resulted in impaired sensory characteristics. Throughout the whole storage period, the control samples had higher sensory scores for all measured attributes than treated samples (**Table 2**).

Table 2. Sensory scores of pork loin samples stored at 4°C.

SMELL

	0	**4**	**7**	**11**	**15**
Control	5^{g}	4.6^{efg}	4.67^{efg}	3.76^{de}	2.98^{abcd}
LDPE	5^{g}	4.68^{fg}	4.71^{fg}	3.88^{def}	2.52^{ab}
FILM B	5^{g}	3.67^{d}	3.38^{bcd}	2.93^{abcd}	2.26^{a}
FILM A	5^{g}	3.48^{cd}	2.98^{abcd}	2.67^{abc}	2.21^{a}

MEAT COLOR

	0	**4**	**7**	**11**	**15**
Control	5^{h}	4.83^{h}	4.64^{gh}	3.86^{ef}	3.19^{de}
LDPE	5^{h}	4.83^{h}	4.43^{fgh}	3.93^{efg}	3.36^{de}
FILM B	5^{h}	2.70^{cd}	2.43^{bc}	2.26^{abc}	1.79^{ab}
FILM A	5^{h}	2.11^{abc}	2.07^{abc}	2.07^{abc}	1.60^{a}

TEXTURE

	0	**4**	**7**	**11**	**15**
Control	5^{i}	4.61^{hi}	4.29^{ght}	3.64^{efg}	2.79^{cde}
LDPE	5^{i}	4.53^{ghi}	4.43^{ght}	3.77^{fgh}	2.95^{cdef}
FILM B	5^{i}	2.97^{cdef}	2.57^{bcd}	2.24^{abc}	1.49^{a}
FILM A	5^{i}	3.28^{def}	2.50^{bcd}	2.24^{abc}	1.79^{ab}

OVERALL

	0	**4**	**7**	**11**	**15**
Control	9^{f}	7.7^{f}	7.74^{ef}	6.31^{de}	4.79^{c}
LDPE	9^{f}	7.66^{def}	7.89^{f}	6.30^{d}	4.19^{bc}
FILM B	9^{f}	4.22^{bc}	4.19^{bc}	3.93^{bc}	2.43^{a}
FILM A	9^{f}	3.45^{abc}	3.26^{ab}	2.98^{ab}	2.31^{a}

Moreover the samples wrapped in developed films were deemed unacceptable in terms of color and overall acceptability after just 4 days, in terms of texture after 7 days and in terms of aroma after 15 days. At the same time both control samples were considered acceptable until day 15 of storage. All studied films caused, aside from drying, the discoloration of meat, which took color characteristics of the active ingredient present in the films.

IV. CONCLUSION

The films successfully inhibited microbial growth, however they also caused deterioration of sensory properties and therefore cannot be used for high-water containing product Surprisingly, the films did not inhibit fat oxidation of the samples, measured through TBARS method. Moreover, the application of FILM A and FILM B seemed to promote the TBARS formation.

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Influence of the shower treatment on the PAH levels in Frankfurters after smoking

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I. INTRODUCTION

Smoking is a traditional method of food preservation that also enhances the sensory properties of smoked meat products (*e.g.* Frankfurters) [1]. In order to produce the expected smoldering smoke, the pyrolysis temperature of woodchips is usually set in the range of 300 to 900 °C. However, it is well known that the polycyclic aromatic hydrocarbons (PAHs) are formed during the incomplete combustion of wood above 500 °C and then accumulate on the surface of smoked meat products. Considering that modern smoking systems involve not only smoking but also many other processes such as drying, cooking, and showering. To extend the shelf life, cooked sausages are cooled down immediately in the smoking chamber by showering them with cold water. The aim of this study was to verify the hypothesis that showering significantly affects the levels of PAHs in sausage casings.

II. MATERIALS AND METHODS

Production of Frankfurters: The beef, pork, pork back fat, sheep casings and additives were purchased from MEGA eG (Stuttgart, BW, Germany), spices from Frutarom Production GmbH (Freilassing, BY, Germany), and beech woodchips "Räuchergold KL 2-16" from J. Rettenmaier & Söhne GmbH+Co KG (Rosenberg, BW, Germany). The Frankfurters were produced as described [1]. The smoke was produced by a temperature-controlled smoldering smoke generator (REICH Thermoprozesstechnik GmbH, Schechingen, BW, Germany). The woodchips were ignited in the first 132 s, and then kept smoldering at two set temperatures (750 °C and 900 °C) by adjusting the volume flow. The specific parameters of the whole processing are shown in Table 1. The casings were removed form sausages for the determination of PAHs. A three-way-ANOVA was used as a statistical test.

Table 1 – The specific parameters of Frankfurters production.

Parameter	Production process				
	Reddening	Drying	Smoking	Cooking	Showering
Temperature/ time	55 °C/30 min	50 °C/12 min	750 °C/3,11,15 min 900 °C/3,11,15 min	75 °C / 20 min	Ca. 12°C 10 min
Humidity	80%	/	/	97%	/
Fresh air	/	100%	/	/	/
Exhaust	/	100%	75%	/	50%
Circulating air in smoke chamber	12.2 m^3/h	12.2 m^3/h	17.4 m^3/h, 900 °C 16.0 m^3/h, 750 °C	14.0 m^3/h	5.2 m^3/h
Transportation air and smoldering air	/	100%	100%	/	/

Determination of PAHs: PAHs calibration mix standard was purchased from Sigma-Aldrich Co. Ltd (Laramie, WY, USA). The Sep-Pak silica cartridges of Waters (Milford, MA, USA) were used. The extraction, clean-up and determination of PAHs in casings was carried out according to our established method [1].

III. RESULTS AND DISCUSSION

Although, the cold-water showering rapidly cools down the core temperature of cooked Frankfurters for a longer shelf life, the impact of this treatment on the contents of PAHs is still unclear so far. As illustrated in Figure 1, the residual levels of PAHs were significantly influenced by the smoke generation temperature and the smoking time ($p<0.001$). In contrast, the levels of both benzo[α]pyrene (BαP) and PAH4, as well as the content of each PAH, showed only slight differences ($p>0.05$) between the casing samples of before and after showering under the same smoking conditions. It should be noted that the weight ratio of the casing to the whole sausage was only approximately 5.78 wt%, and the levels of PAHs in the meat were much lower. Therefore, the levels of BαP and PAH4 in Frankfurters did not exceed the maximum levels specified in the EU Regulation (Commission Regulation (EU) 2023/915) for smoked meat products, which are 2.0 µg/kg for BαP and 12.0 µg/kg for PAH4.

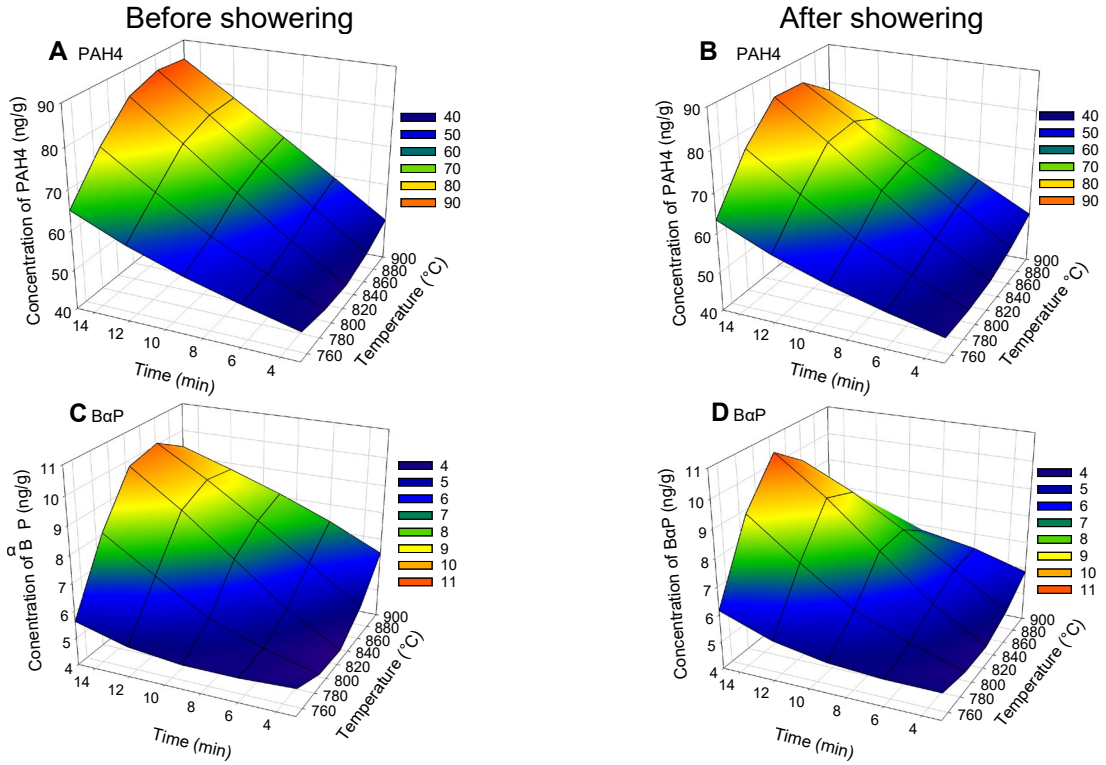

Fig 1. PAH4 and BαP levels before and after showering as a function of smoke time and generation temperature

IV. CONCLUSION

The results of this study showed that PAH levels were significantly affected by smoke generation temperature and smoking time. However, showering had only a slight effect on the accumulation of BαP and PAH4 in sausage casings. These findings suggest that PAHs may be incorporated or bound into the matrix of natural casings, which cannot be mitigated by cold-water showering.

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INACTIVATION OF TOXOPLASMA GONDII IN MEAT SAMPLES

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I. INTRODUCTION

Toxoplasma gondii is one of the most important zoonotic parasites in the world and virtually all warm-blooded animals, including humans, mammals and birds can be infected by this pathogen (Dubey, 2009). Globally, *T. gondii* is ranked fourth out of 24 foodborne parasites (World Health Organization, 2015). *T. gondii* is the causative agent of toxoplasmosis in humans. Pregnant women and severely immune-compromised persons are at risk for serious complications due to congenital transmission or disseminated infection. Consumption of raw and undercooked meat is considered as an important source of *T. gondii* infections. However, most non-heated meat products contain salt and additives, which affect *T. gondii* viability. The mouse bioassay is the standard method for testing of samples on viable *T. gondii* parasites, but ethically undesirable, costly and time consuming. It was our aim to develop an in vitro method to substitute the mouse bioassay for determining the effect of salting on *T. gondii* viability.

II. MATERIALS AND MTHODS

Processing experiments were performed with minced meat from sheep that were experimentally infected with *T. gondii* oocysts (Opsteegh et al. 2024). Portions of 50 g infected sheep meat were supplemented with 0.6 - 2.7 g NaCl/100 g. In addition, a series of 1.2 g NaCl/100g was set up supplemented with 1.4 or 1.8 g sodium lactate/100g and one combination with 1.2 g NaCl/100 g supplemented with 1.4 g sodium lactate and 0.4 g sodium acetate/100g of sheep meat. Besides those samples, untreated sheep meat, *T. gondii* free meat and frozen sheep meat samples were used as control samples. All meat samples were tested twice and in duplicate, some even in quadruplicate. The cell culture method (Opsteegh et al. 2024) and the mouse bioassay were used to determine effect of salting on the viability of *T gondii.*

III. RESULTS AND DISCUSSION

The results with the different salt concentrations showed differences in growth of *T. gondii*. In samples with NaCl concentrations of 1.5 g/100 g or higher, no viable *T. gondii* was detected by cell culture and by mouse bioassay. When sodium lactate (with or without sodium acetate) was added, no viable *T. gondii* was detected at the concentration of 1.2% NaCl or higher. The cell culture method showed the same results as the mouse bioassay (Tables 1 and 2).

Table 1 – Detection of *T. gondii* by cell culture and by mouse bioassay in infected sheep meat supplemented with different salt concentration after 20 h incubation.

	Test week 1		**Test week 2**	
Duplicate samples	Cell culture	Mouse bioassay	Cell culture	Mouse bioassay

Negative	-	-	-	-
Positive	+	+	+	+
0.6 -0.9 g NaCl/100 g	+	+/-	+	+
1.2 g NaCl/100 g	-	-	+	+
1.5 -2.7 g NaCl/100 g	-	-	-	-

+ means growth on both duplicates +/- means growth on one of the duplicates
- means no growth on both duplicates

Table 2 – Detection of *T. gondii* by cell-culture method and mouse bio assay in infected sheep meat supplemented with different concentrations of additives after 20 h incubation.

	Test week 3		**Test week 4**	
quadruplicate samples	Cell culture	Mouse bio assay	Cell culture	Mouse bio assay
Negative	-	-	-	-
Positive	+	+	+	+
1.2 g NaCl/100 g	+	+	+	+
1.2 g NaCl + 1.4 g Na-lac/100 g	-	-	-	-
1.2 g NaCl + 1.8 g Na-lac /100 g	-	-	-	-
1.2 g NaCl + 1.4 g Na-lac + 0.4 g Na-ac /100 g	-	-	-	-

- means no growth on the quadruplicates, + means growth on the quadruplicates

IV. CONCLUSION

The level of NaCl concentration present in meat products affects the viability of *T. gondii* in minced meat. With the addition of Na lactate, negative effects on *T. gondii* were obtained at a lower NaCl concentration. The developed cell culture method can successfully be used to detect viable *T. gondii* in tissues of experimentally infected sheep and replace mouse bioassay in these types of inactivation experiments.

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RAPID DETECTION OF TUBERCULOSIS IN BEEF CARCASSES

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I. INTRODUCTION

Bovine tuberculosis (TB) is a chronic bacterial disease caused by microorganisms from the *Mycobacterium tuberculosis* complex, mainly *M. bovis*. Many batches of cattle are disqualified for export due to suspicion of TB established during visual, ante- and post-mortem inspections, carried out during the slaughter of cattle, by the official Brazilian Veterinary Inspection Service (SIF) without proper laboratory confirmation. Therefore, disabling batches of animals for export based exclusively on visual observation is beyond the modern sense of inspection, economy, and sustainability. Fast, sensitive, and specific analytical techniques, without the need for laboratory structure and trained technical personnel, are available for the diagnosis of human tuberculosis and are approved for use by government health agencies (Brasil, 2022). The objective of the present study was to apply the qPCR technique, using the GeneXpert equipment and the MTB/RIF kit (GXMTB), on samples collected directly from lesions on carcasses suspected of TB defined by the SIF. The results for detection of *M. tuberculosis* Complex agents, obtained from this new sampling matrix, were compared with those from microbiological culture (MC), considered the gold standard method.

II. MATERIALS AND METHODS

100 samples were analyzed, 94 originating from lesions suspected of TB and six from lesions considered non-specific by the Federal Inspection. Initially, the samples were collected with a swab from the injured organ/tissue and placed in a conservative solution provided in the GXMTB kit, immediately applying the protocol indicated by the manufacturer (Cepheid®, 2020). Samples of 1g were taken from the same injured organs/tissues, placed in an isothermal box containing ice and sent to the laboratory of the Department of Preventive Veterinary Medicine and Animal Health at FMVZ-USP, where they were inoculated in Lowenstein-Jensen and Stonebrink culture media. DNA was extracted from typical colonies and conventional PCR was applied to differentiate *Mycobacterium* spp., *M. tuberculosis* and *M. bovis*. The lesions were also subjected to histopathological and cytological analysis. The results obtained through the GXMTB protocol and MC were evaluated using the McNemar and Kappa statistical tests to determine agreement and replicability between them.

III. RESULTS AND DISCUSSION

The results of the analysis with the GXMTB kit were obtained, on average, 77 minutes after sampling. When comparing the results from GXMTB and MC methods by the McNemar test, no

disagreement was observed between them (p=1). However, there was disagreement between the SIF and MC results ($p < 0.0001$) and between SIF and GXMTB ($p = 0.0034$). The application of the Kappa test indicated that the replicability was considered as “GOOD” when comparing the results between the GXMTB and the MC ($p < 0.0001$), and replicability was considered “BAD” when comparing the other analytical methods investigated ($p < 0.0001$). Histopathology and cytology confirmed lesions characteristic of TB, as well as the presence of acid-alcohol resistant bacilli. All isolates were confirmed by conventional PCR as *M. bovis* and all of them didn’t show Rifampicin resistance.

Table 1 – Results of 100 samples processed after veterinary inspection showing agreement and disagreement between the three methods applied.

Results	+ +	+ -	- +	- -	Total
GXMTB / SIF	82	1	12	5	100
GXMTB / MC	77	6	6	11	100
SIF / MC	81	13	2	4	100

Handling the samples, as well as performing the analyzes with the GXMTB kit, were extremely fast and easy, providing robust results without the need for specialized laboratories or highly trained personnel, especially when compared to traditional methods that take up to 100 days. The short time required -less than 90 minutes- to obtain results allows confirmation of suspected TB even when the meat has not completed sanitary maturation, providing an extremely useful tool for determining destination markets.

IV. CONCLUSION

The GXMTB analytical method showed agreement/replicability with the MC (Gold Standard) for detection and identification of *M. bovis* and was efficient when applied from a new sample matrix. It was extremely fast and easy to handle, providing robust results without the need for specialized laboratories or highly trained personnel, especially when compared to traditional methods that take up to 100 days. Therefore, it can be used as an auxiliary method in the immediate diagnosis of TB. The precision and speed in obtaining results by GXMTB allowed TB confirmation when the carcass has not yet completed its sanitary maturation, thus enabling safety decisions regarding the destination of the carcasses and the qualification/disqualification of the animals’ batch with exportation potential. Only *M. bovis* was detected and none of them showed Rifampicin resistance.

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BACTERIAL TRANSLOCATION DURING DELAYED EVISCERATION

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I. INTRODUCTION

During cattle slaughter, carcass evisceration may be postponed. There is a belief that these delays can lead to contamination of carcasses by bacterial invasion, mainly from the contents of the digestive tract (1). A study was carried out to assess the consequences of delayed evisceration (DE) of cattle. The effect of DE, two hours after stunning and bleeding, was evaluated regarding possible bacteriological, physicochemical, and sensory changes capable of impacting the quality of the animals' carcasses and edible offal.

II. MATERIALS AND METHODS

The experiment was carried out with feedlot cattle, slaughtered under federal inspection (SIF) and laboratory analyzes were performed in a laboratory accredited by the Ministry of Agriculture - Brazil. The animals in the experimental group were stunned and bled, waiting hung for two hours before skinning and evisceration began. Those in the control group were processed according to the plant's routine. Hot carcasses and half-carcasses after cooling, perirenal and cavitary fat as well as viscera, were examined assessing general appearance, color, odor, consistency, and shine. pH and temperature of the carcasses, as well as the plant environmental temperature were also measured. Samples for microbiology were obtained from four different points on the external surface and two points on the internal surface (thorax and abdomen) of the half carcasses by smearing sterilized sponges and using a 100 cm^2 template. Using sterilized instruments, a sample of approximately 250 g of heart and liver was collected from each animal from the experiment. By rubbing sterilized sponges and using a 100 cm^2 template, a sample was collected from the external surface of the rumen of each animal. All samples were subjected to the following microbiological counts: aerobic or facultative mesophilic microorganisms; *Enterobacteriaceae*, lactic acid bacteria and mesophilic anaerobic microorganisms.

III. RESULTS AND DISCUSSION

According to the sensory aspects evaluated: general aspect, brightness, color and odor, no differences were observed among the carcasses from the control and the experimental groups, all of which remained within similar standards and considered normal. Likewise, no difference was evident among the viscera of animals from both groups, regarding the consistency and degree of friability of the viscera. After cooling for 24 h, there was no statistically significant difference in the pH of the two groups. According to the microbiological criteria adopted, all bacterial count results remained within the limits considered acceptable (Table 1).

Official veterinary inspection services in Brazil consider DE after 45 minutes of the animal's death. The applied criteria are conditional use of the carcass and the condemnation of the viscera after 60 minutes, and the total condemnation of the viscera and carcass after 90 minutes. There are laws, such as São Paulo's State, which report a time of 40 minutes to DE (2).

Table 1 – Medians (CFU/cm^2), for sampled areas. CG: Control group; EG: Experimental group.

Microorganisms	External surface		Internal surface				Whole carcass results	
			Torax		Abdomen			
	CG	EG	CG	EG	EG	CG	EG	CG
Mesophilic	235a	945b	15a	55a	4a	1a	211a	517b
Enterobacteriaceae	1a	2a	1a	1a	1a	1a	1a	1a
Lactic Acid Bacteria	3.5a	4.5 a	1a	1a	1a	1a	1a	1a
Anaerobic mesophilic	12a	12a	1a	4a	1a	1a	1a	2a

Numbers followed by the same letter at the same line means no statistical difference.

Our results showed no sensory and physicochemical changes or abnormalities that would justify condemning the carcasses and viscera from the experimental group (two-hour DE) not violating the requirements from RIISPOA Arts. 129, 142 and 143 regarding DE episodes (3). Bacteriological evaluation did not reveal values above the microbiological limits established in any of the analytical results (Table 1). The median counts of mesophilic microorganisms obtained in samples from the peritoneal surface were extremely low, with values close to 10^0. Therefore, this does not demonstrate the occurrence of bacterial translocation after two hours. This period also did not impact the microbiological quality of the edible viscera.

IV. CONCLUSION

It can be concluded that DE, with up to two hours for starting skinning and evisceration, did not negatively impact the acceptable sensorial, physicochemical, and microbiological quality of fresh meat and edible offal from slaughtered cattle.

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MOLECULAR DETECTION OF NON-VIABLE SARS-COV-2 IN FROZEN MEAT PACKAGING

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I. INTRODUCTION

Faced with the rapid spread of SARS-CoV-2, in March 2020 the World Health Organization (WHO) issued a pandemic alert. According to Official Letter No. 09199.000448/2020-80, health control measures became mandatory to minimize the risk of transmission of the SARS-CoV-2 virus, since the Chinese health authorities adopted the testing of packaging for detection of genetic material from this virus. This detection on packaging can generate the cancellation of export authorization (1). The sanitization methods used to inactivate the virus, should also cause some degree of denaturation and/or structural disintegration of the viral RNA to prevent detection during sampling performed by Chinese sanitary authorities. However, commercially available sanitizers seemed not to have demonstrated effectiveness in inhibiting the amplification of the viral genome by RT-qPCR, resulting in several frozen-meat containers being rejected and returned to the exporter. Apparently, the use of these products still results in the detection of viral genomic copies, despite the virus no longer being viable and, therefore, no longer contagious/infectious.

The objective of this project was to evaluate the influence of potentially interfering substances (sodium hypochlorite 2%, 3% and RNAse) on the amplification of gene fragments present on the surface of secondary packaging (cardboard) used for frozen meat cuts.

II. MATERIALS AND METHODS

The secondary packaging (cardboard), from two different commercial brands used for shipping frozen meat were cut into 2cm x 2cm squares and then sprinkled with 100 µL of a nasopharyngeal swab sample containing inactivated SARS-CoV-2 with a viral load of E+06/µL and stained with Trypan Blue. Additionally, the same procedure was performed using the commercial SARS-CoV-2 genome 2019-nCov_N_Positive Control (IDT) with a viral load of E+05 genomic copies/uL stained with Trypan Blue as an internal process control. The cardboard squares (5 replicas of each type - A and B) were sprayed with 100µL of sanitizing solutions of 2% and 3% sodium hypochlorite, RNAse (Nuclease P1) and PBS (control), placed in a petri dish (1 dish for each cardboard square), vacuum packed and stored at -20ºC for 24h and 30 days, respectively. Each treatment was carried out with five repetitions on aliquots of cardboards A and B sprinkled with a sample with inactivated SARS-CoV-2, in addition to the negative control (PBS) and the internal process control (commercial SARS-CoV-2 commercial genome - CIP). For viral genomic detection, the samples were left for 1 h at room temperature in a laminar flow chamber. Next, the samples were sonicated, the viral genetic material extracted using Purelink Viral RNA/DNA Mini Kit (Invitrogen) and RT-qPCR performed.

III. RESULTS AND DISCUSSION

Analysis of the RT-qPCR results demonstrated that the viral genome was detected in all cardboard aliquots (both A and B), before and after the different treatments carried out. Analysis of external

cardboard samples sprayed with inactivated virus (E+06 genomic copies/mL) and subjected to treatments with 2%, 3% sodium hypochlorite and RNAse, after 24h, demonstrated an average viral load of 1.44E+03, 5.88E+03 and 2.39E+03, respectively. The mean values of the negative control (PBS treatment) and CIP were 9.13E+04 and 1.69E+04, respectively. The means found for treatment analyzes after 30 days of freezing the material were similar: 8.55E+04, 1.34E+05 and 1.31E+04, for treatments with sodium hypochlorite 2%, 3% and RNAse, respectively. The means for the negative and positive controls were 9.13E+04 and 1.31E+04, respectively. Regarding the analysis of internal cardboard samples after 24h, the average viral genomic load after treatment with 2%, 3% sodium hypochlorite and RNAse were 5.73E+04, 5.07E+03 and 9.56E+04, respectively. The mean values of the control and CIP were 1.18E+05 and 1.67E+04, respectively. As observed in the analysis of the external packaging, the mean viral load values for treatments after 30 days showed similar values. Treatments with 2%, 3% sodium hypochlorite and RNAse showed mean viral load values of 1.61E+05, 9.91E+03 and 3.33E+04, respectively. The mean values of the control and CIP were 2.27E+05 and 1.48E+04, respectively. The results demonstrate a loss of approximately 1 log of inactivated virus and commercial viral genome during material processing. An analysis of variance was carried out for statistical evaluation and comparison of the effect of treatments in the two packages, for the two incubation periods (24h and 30 days). After incubation at -20ºC for 24 hours, package A showed a reduction in viral load with the 3 treatments and better efficiency was observed with 2% Hypochlorite or RNAse, while package B showed a reduction in viral load only with 3% Hypochlorite treatment. Considering incubation for 30 days, packages A and B showed a reduction with RNAse treatment, however package B showed better efficiency after treatment with 3% Hypochlorite. Sodium hypochlorite in used concentrations is known to be capable of inactivating SARS-CoV-2 (2). However, genomic detection continues as nucleic acid fragments remain available originating false positive results.

IV. CONCLUSION

None of the potentially interfering substances tested were able to prevent viral-RNA detection in any of the project's treatment conditions. Although unable to cause infection due to the viral inactivation process, fragments of viral genetic material persist. This allows to detect viral-RNA using different molecular techniques. It means that molecular methods performed by sanitary authorities to prevent transmission of SARS-Cov-2 were not suitable, as they may have been identifying just RNA viral fragments of a non-viable and non-infectious pathogen, which has no risk for public health. Thus, molecular methods should be followed by viral culture of sampled material to confirm the presence of viable viruses and the real risk of infectious transmission. These findings could support the criteria for future international trade agreements.

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EFFICACY OF DIFFERENT ANTIMICROBIALS TREATMENT ON CHICKEN CARCASS DECONTAMINATION

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I. INTRODUCTION

During poultry slaughtering carcasses are washed with water on the slaughter floor, under pressure, after weighing and before chilling to minimize microbial contamination. Various chemical agents are added to the final rinse and have been used as antimicrobials for carcasses decontamination over the years [1,2]. These include short chain organic acids, chlorine washes, tri-sodium salts, etc. However, the efficacy of these antimicrobials is still inconclusive [1]. It depends on several factors such as concentration and contact time of antimicrobial solution with chicken samples, the level of attachment of bacterial infection on the chicken skin, method and technology used [3,4]. Hence the aim of this study was to determine the effectiveness of chlorine bleach, tri-sodium citrate, lactic acid, and acetic acid on the microbial populations on chicken carcasses decontamination. These antimicrobials are affordable, and easily accessible to be used by small scale poultry farmers who have limited knowledge and technology.

II. MATERIALS AND METHODS

Chickens were slaughtered at the mobile abattoir owned by small-scale poultry farmer. Ten chicken carcasses were collected immediately after the last rinse that was free of standard decontamination chemical. All chicken carcasses were individually placed in ziploc bags, placed into cooler boxes filled with ice and immediately transported to the Onderstepoort Veterinary Research bacteriology laboratory for microbial analysis. The consensus of the research is that carcass decontamination can reduce the initial levels of bacteria on the surface of the carcasses [1]. Therefore, the chicken skin was used for this experiment. Upon arrival of the chicken carcasses in the laboratory, the skin was removed aseptically from different parts of each of ten broiler carcasses. It was cut into small pieces with sterile scissors. Twenty-five (25) grams of each sample was added into 225 mL of sterile buffered peptone water and four different treatment solutions which included i) Chlorine bleach (0.005 %), ii) Trisodium citrate (2.0 %), iii) Lactic acid (2.0 %), and iv) White vinegar (5.0 % acetic acid) and homogenized in a stomach bag for one minute at speed 6. Further tenfold serial dilutions were made with sterile buffered peptone water. Duplicate plates were made for each sample at each dilution under ISO 6887–1: 2017 standard methods and incubated at 37 °C for 24hrs. Aerobic bacteria were enumerated and expressed as Colony-Forming Units per gram (cfu/g). The mean microbial counts were converted into base-10 logarithms of cfu/g. All data were subjected to an analysis of variance (ANOVA) to test for significant treatment effects using GenStat for Windows 22nd Edition [5]. Fisher's protected t-LSD (Least Significant Difference) was calculated to compare means of significant effects at the 5% level.

III. RESULTS AND DISCUSSION

The results of microbial loads expressed as log cfu/g are presented on Figure 1. Acetic acid treatment had the lowest ($P<0.001$) aerobic plate counts compared to other antimicrobials used in the study. Chlorine bleach, trisodium and lactic acid treatments had the highest bacterial counts (4 log cfu/g),

however the counts were still within the acceptable upper limits of 6 log cfu/g in foodstuffs, according to the South African regulations governing microbiological standards and food stuffs and related matters, Government notice, No. R.692 [6]. Although the concentrations and contact time of the sample with antimicrobial used in this study have been proposed, tested and approved for their use in decontamination systems [2], it is hard to make a direct comparison in concentrations or time. However, it would be assumed that the 5 % acetic acid was more effective in reducing microbial populations than other antimicrobials with lower concentrations within the experiment dimensions. In poultry carcasses decontamination, the amount of concentration and contact time of antimicrobial solution with chicken samples determines the effectiveness of the disinfectant [3,4].

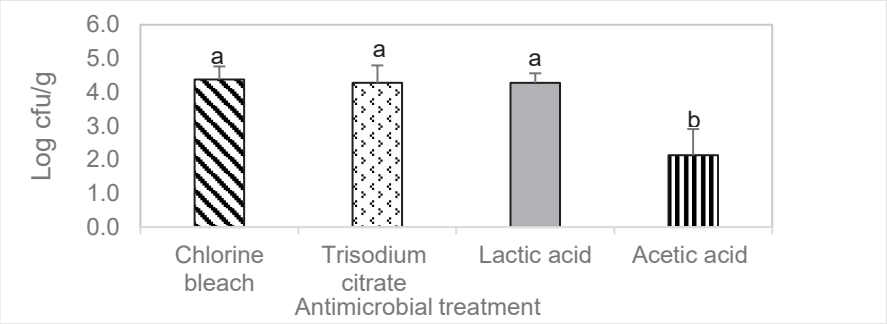

Figure 1. Total Aerobic Plate Counts of each sample expressed as log cfu/g

Means with different superscripts in the same row differ significantly (P<0.05).

IV. CONCLUSION

It is concluded that white vinegar (5% acetic acid) is an effective disinfectant and can be used as an alternative antimicrobial to a well known antimicrobials (chlorinated water, acetic acid and tri-sodium salts) for reduction of the bacterial population of poultry carcasses. Its effectiveness, affordability and accessibility makes it a better preservative for chicken, especially for small scale farmers with limited knowledge and technology in the application of these antimicrobials. However, further studies should be done to ascertain its effectiveness on specific micro-organims commonly associated with fresh chicken.

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A PRELIMINARY ATTEMPT TO CULTIVATE AWARENESS OF ALPHA-GAL SYNDROME IN RURAL KANSAS AND ASSESS CONCENTRATIONS OF ALPHA-GAL IN VARIOUS MEAT PRODUCTS

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I. INTRODUCTION

Alpha-gal syndrome (AGS), also known as red meat allergy, is caused by lone star tick (*Amblyomma americanum*) bites. The tick introduces galactose-alpha-1,3-galactose (α-Gal) into the bloodstream, triggering a surge in specific IgE antibodies against α-Gal. The allergic reaction typically occurs 2-6 hours after the consumption of food products containing mammalian tissues, and the symptoms can range from hives, swelling and gastrointestinal distress to potentially life-threatening reactions such as respiratory difficulties and anaphylaxis. Historically, AGS was most prevalent in the southeastern US, where the lone star tick population is well established. However, the tick population range has expanded in the US, and an increased number of AGS cases has been reported in Kansas in the recent years. Unfortunately, little is known about the AGS prevalence throughout Kansas. Furthermore, no work has been conducted to quantify the amount of α-Gal present in different meat products to evaluate the potential impact of processing technique on α-Gal concentration. Thus, the two objectives for this study are: 1) to gain a better understanding of the prevalence of AGS in rural Kansas communities; and 2) to quantify the α-Gal concentration in various fresh and processed meat products that are commonly consumed by Midwesterners.

II. MATERIALS AND METHODS

For the first objective, 160 incentivized surveys were sent out to custom-exempt, state and federally inspected meat processors across the state of Kansas. Survey questions were designed to understand AGS prevalence in Kansas and the respondents' prior knowledge of AGS. For the second objective, 10 beef striploins and 10 batches of 8 different processed meat products were purchased. The strip loins were either left raw or cooked to medium rare (MR; 54℃) medium (MED; 60℃), or well done (WD; 70℃). The processed meat samples were fully cooked pork bratwurst, fully cooked bacon, deli ham, beef hot dog, classic hot dog (mostly chicken, but with pork and beef added), beef summer sausage, beef jerky, and fully cooked beef hamburger patty. Whole muscle protein was extracted from each sample and separated by gel electrophoresis and immunoblotted against anti-α-Gal IgG1. Additionally, each gel included a reference sample of α-Gal Conjugated-Human Serum Albumin (HSA) with a known α-Gal content of 59.2 pmol. The concentrations of α-Gal in the samples were determined as the ratio of the lane densities of the sample and the HSA reference.

III. RESULTS AND DISCUSSION

Of the 160 sent surveys, 50 were returned. Survey results showed that 28% of respondents knew at least one person in their area with AGS. The geographical distribution of these cases was concentrated in the south central and southeastern regions of Kansas, which is consistent with the range of the lone star tick (figure 1). Furthermore, only 58% of respondents knew that red meat allergy is related to tick bites and 96% of them expressed there is not enough public information about AGS. On the other hand, it was clear that fresh beef striploin steaks had much higher α-Gal concentration than processed meat products regardless of degree of doneness ($P < 0.01$). It was interesting to note that α-Gal concentration increased as the degree of doneness increased for beef striploins ($P < 0.01$), with the lowest concentration in raw sample (8.24 pmol/μg protein), followed by MR and MED (11.09 and 10.85 pmol/μg protein, respectively), with the highest concentration in WD samples (13.05 pmol/μg protein). Among the processed meat products, it was determined that pork brats and beef hot dogs had the

highest concentration of α-Gal with 3.29 and 3.26 pmol of α-Gal/µg of protein, respectively, followed by cooked beef patty with 2.76 pmol of α-Gal/µg of protein, beef jerky at 2.45 pmol/µg of protein, cooked bacon with 2.11 pmol/µg of protein, beef summer sausage with 1.77 pmol/µg of protein, deli ham with 1.37 pmol/µg of protein, with classic hot dog having the lowest concentration of α-Gal at 0.88 pmol per µg of protein ($P < 0.01$; figure 2).

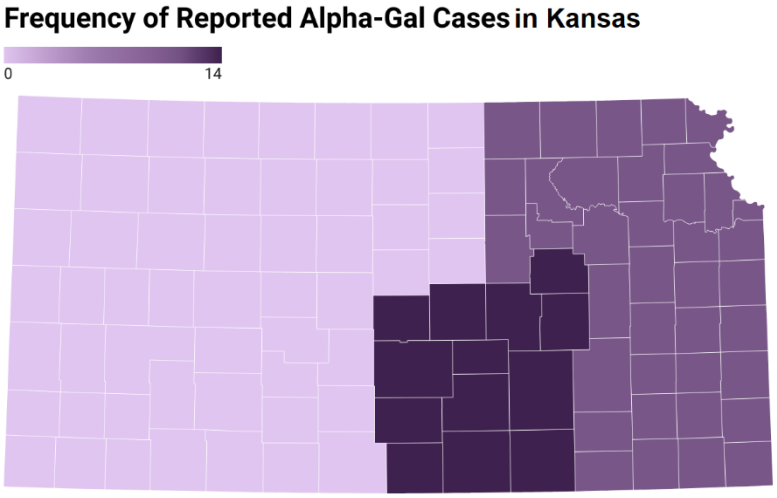

Figure 1. Prevalence of AGS in Kansas, USA based on the 160 surveys sent to small meat processors in Kansas.

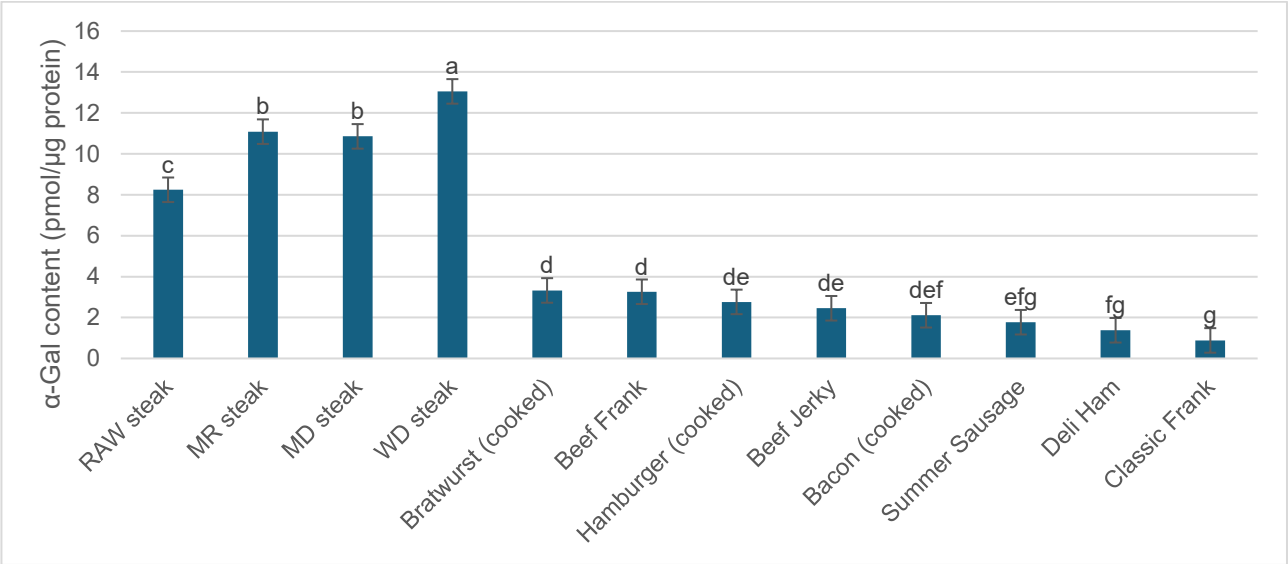

Figure 2. The α-Gal concentration of beef striploin steaks cooked to various degree of doneness [raw, medium rare (MR), medium (MD) and well done (WD)] and various processed meat items.
[a-g] Least sqaure means without a common superscript differ from each other.

IV. CONCLUSION

The AGS is a growing clinical and public health concern for people in the US and the world, which AGS has been reported to occur in at least seventeen nations worldwide. Our preliminary survey results indicated a need for AGS education in Kansas, especially amongst rural communities and those located in geographically high-risk areas. On the other hand, our α-Gal content data suggested that α-Gal content could be influenced by freshness, cooking methods, species, use of collagen casing and other processing techniques, while heat itself does not reduce α-Gal concentration. Future research should focus on determining the relationship between α-Gal content/consumption level and blood α-Gal IgE antibody production in AGS patients for better management of the condition.

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Effect of UV pulse light on the growth and resistance of *Pseudomonas* spp.

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I. INTRODUCTION

Pseudomonas spp. are aerobic, gram-negative bacteria that can be isolated from diverse environments, such as water, soil, plants, and animals. *Pseudomonas* spp. besides being a spoilage indicator in meats, can also be opportunistic pathogens, namely *P. aeruginosa* that causes nosocomial and community-acquired lung infections [1,2,3]. Additionally, due to overuse and misuse of antibiotics, *Pseudomonas* spp. have become increasingly more resistant, especially to the carbapenem class of antibiotics and to colistin. Colistin is an antibiotic frequently used as a last resort to treat infections by multidrug-resistant and extensively drug-resistant *Pseudomonas* spp., thus the increase in resistance is very concerning [1,2,4]. This increase in resistance coupled with the extensive presence of these bacteria in the food chain increases the possibility of cross-contamination[1,2]. As such it is vital to find new methodologies to control and eliminate these bacteria from the food processing environment. The UV pulse light technology is an emergent and non-thermal process, generally considered as a surface decontamination technology, but its effectiveness is dependent on several factors [5,6,7]. The aim of this work was to evaluate the influence of a UV pulse light treatment on the growth of carbapenem and colistin-resistant *Pseudomonas* spp. versus the effect of the treatment on the growth of susceptible *Pseudomonas* spp.. In addition, the effect of the treatment on the resistance profile of the strains was also evaluated.

II. MATERIALS AND METHODS

Two *Pseudomonas* spp. isolated from the pork meat chain were utilized, one resistant to meropenem and colistin, the OS1S E9 (*Pseudomonas lactis*), and one susceptible to both antibiotics, TSB5 (*Pseudomonas fragi*). Both species belong to the Laboratory of Food Technology collection of the Faculty of Veterinary Medicine (University of Lisbon). To test the UV light treatment on simulated early biofilm formation on food-grade surfaces, both strains were inoculated, to a concentration of 10^8 bacteria/cm^2, in one square centimeter of food-grade stainless steel surface finish category 2B and left to dry overnight at room temperature. This was done to simulate a *Pseudomonas* biofilm on a food-processing surface. After the incubation, the inoculated discs underwent the UV pulse light treatment. For each strain four different treatments were tested, the control (no treatment), treatment A (2.582 J/cm^2), treatment B (4.303 J/cm^2), and treatment C (7.746 J/cm^2). After treatment, the bacteria were removed from the discs through sonication and vortex agitation with glass beads. The bacteria were then inoculated in *Pseudomonas* CFC medium (Scharlau, Spain) according to ISO 13720:2010. After enumeration, five colonies of each isolate and treatment were recovered to study their antimicrobial profile. The antibiotics tested were meropenem and colistin, utilizing the e-test methodology (Biomerieux, France). The control utilized was *E. coli* ATCC 25922.

III. RESULTS AND DISCUSSION

The effect of the UV pulse light treatments on the two isolates can be seen in Table 1. Overall, for both *Pseudomonas* spp. the treatments were shown to be significantly more effective when in comparison with the control ($\alpha \leq 0.05$). For the strain OS1S E9 the most significantly effective treatment was treatment C (7.746 J/cm^2) which had the highest fluence. The TSB5 isolate was more affected by treatment B,

however it was not significantly different from treatment C (α>0.05). Additionally, it can be seen in treatment B and treatment A, that the resistant strain OS1S E9 was significantly less susceptible to the treatments than the antibiotic susceptible strain TSB5. The results demonstrate that while all treatments had a high bactericidal effect (>5 Log), treatment C was the most effective overall *Pseudomonas* spp..

Table 1. Average values for the enumeration in Log cfu/cm^2 for each treatment and the two *Pseudomonas* spp. OS1S E9 and TSB5.

Strains	Control	Treatment A	Treatment B	Treatment C
OS1S E9	5.77^a	2.31^b	1.55^{b,c}	0.00^d
TSB5	5.09^a	1.31^c	0.34^d	0.69^{c,d}

a,b,c,d – average values followed by different letters represent significant differences (α<0.05). To facilitate the statistics the limit of detection <1 log cfu/cm^2 was assumed as zero.

In this study besides the bactericidal effect of the treatment on the bacteria, it was also evaluated the effect of the treatment on the antibiotic profile of the strains. As mentioned above, the OS1S E9 before treatment was found to be resistant to meropenem and colistin while the TSB5 was found to be susceptible to these antibiotics. After the treatments the recovered colonies were tested for their antibiotic resistance profile against the two antibiotics. It was found that TSB5 isolates recovered after the highest treatment, treatment C, were found to have gained resistance to meropenem. In contrast, the OS1S E9 isolates recovered after treatment A and B were found to be susceptible to colistin. These preliminary results indicate a potential influence of the UV pulse light treatment on the resistance of the bacteria.

IV. CONCLUSION

The results present in this work demonstrated the capacity of this new technology, the UV pulse light, to control *Pseudomonas* spp. in conditions like the food processing environment. The highest treatment with a fluence of 7,746 J/cm^2 demonstrated to be effective independently of the resistance profile of the strains. However, this same treatment led to the appearance of resistance to meropenem in the recovered strains, which can be concerning since it can lead to transmission of resistance in the environment. On the contrary, these preliminary results also demonstrated that the treatment with UV pulse light can also lead to loss of resistance to colistin in resistant isolates. In conclusion, the effect of this emergent technology on the antibiotic profile should be further investigated.

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HYPERBARIC STORAGE EFFECT ON CAMPYLOBACTER CONTROL ON POULTRY

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I. INTRODUCTION

Hyperbaric storage is an emerging technology currently under investigation as an alternative to traditional refrigerated storage for various foods, including meat [1]. Typically, hyperbaric storage is employed in association with vacuum-packaging [2]. Poultry meat is recognized as a primary source of *Campylobacter* spp., a pathogen that has been identified as the leading cause of human gastroenteritis in the EU and elsewhere [3]. This study aims to: 1) evaluate the effectiveness of hyperbaric storage in reducing the survival of *Campylobacter* spp. in non-packaged poultry meat; 2) assess the impact of hyperbaric storage on the control of poultry meat spoilage microbiota, color and lipid oxidation.

II. MATERIALS AND METHODS

To assess the effect of hyperbaric storage on the survival of *Campylobacter* spp., samples of broiler breast steaks (BI), and neck skin (SI) were inoculated with a mixture of two strains of *Campylobacter jejuni* (NCTC 11168 and a wild strain 46E isolated from poultry carcass), to achieve approximately 6 log ufc/g. Non-inoculated samples [broiler carcass (CS), breast steaks (BS) and neck skin (SS] were submitted to hyperbaric storage (0.22 MPa, 100% O_2, 21°C, 12 hours) using the Hyperbaric chamber (HVM™, 6400, USA). Analysis was performed before and after hyperbaric storage. Total aerobic mesophilic bacteria, psychrophilic bacteria, *Enterobacteriaceae, Pseudomonas* spp., and *Campylobacter* spp. counts were determined in accordance with ISO Standards. TBARS and pH were assessed in broiler carcass (CS) and breast steaks (BS). L*a*b* color measurements were obtained for broiler carcass (CS), breast steaks (BS), and neck skin (SS) using a Konica Minolta CR-400/410 instrument with illuminant D65. These assays were repeated on three separate working days.

III. RESULTS AND DISCUSSION

The inoculated breast steaks exhibited a reduction in *Campylobacter* spp. of 0.55 log cfu/g, decreasing from 5.38 log cfu/g before storage to 4.84 log cfu/g after hyperbaric storage. Similarly, in the inoculated neck skin, a reduction of 1.33 log cfu/g was observed, from 5.87 log cfu/g to 4.54 log cfu/g after storage. These results from the inoculated samples highlight the potential effectiveness of hyperbaric storage with 100% oxygen, in reducing *Campylobacter* spp. contamination. Microbiological counts from non-inoculated samples are presented in Table 1. The effect of storage negatively impacted total aerobic mesophilic and psychrophilic bacteria, *Enterobacteriaceae*, and *Pseudomonas* spp. counts on carcass, breast steak and neck skin samples, probably due to the favorable temperature and to the lack of inhibitory effect of oxygen. Even in not inoculated samples the effect of hyperbaric storage resulted in a significant ($p<0.05$) decrease in *Campylobacter* spp. counts, in carcass and neck skin samples. These findings suggest an interesting potential of this preservation method in controlling *Campylobacter* spp., that deserves to be better understood to perceive what is the effect of the pressure and the atmosphere composition, and the indirect effect of the natural microbiota on the pathogen' behavior.

Table 1 – Microbiological analysis results of carcass, breast steak, and neck skin before and after hyperbaric storage (n=3).

Sample	Carcass					Breast Steak					Neck Skin				
Parameter (log cfu/g)	TA	PA	E	P	C	TA	PA	E	P	C	TA	PA	E	P	C
Before Storage	4.0^{a}	4.8^{a}	1.9^{a}	2.6^{a}	1.2^{a}	4.3^{a}	4.3^{a}	2.4^{a}	3.5^{a}	0.5	4.7^{a}	4.8^{a}	3.4^{a}	3.1^{a}	3.1^{a}
After Storage	5.7^{b}	6.4^{b}	4.4^{b}	5.9^{b}	0.2^{b}	7.2^{b}	7.5^{b}	5.7^{b}	6.7^{b}	0.3	7.3^{b}	7.4^{b}	7.1^{b}	6.7^{b}	2.7^{b}

TA: Total Aerobic Mesophilic; PA: Psychrophilic aerobic; E: *Enterobacteriaceae*; P: *Pseudomonas* spp.; C: *Campylobacter* spp.. Means followed by different letters in the same column are different ($p < 0.05$). Values below limit of detection (10 cfu/g) were considered 0, for statistical purposes.

The analysis of variance revealed that hyperbaric storage had no significant effect on the pH of breast steaks, (pH=5.9) observed before and after treatment. For carcasses, the effect of hyperbaric storage on pH was significant ($p < 0.001$), still small in dimension, from 5.9 before to 5.8 after storage. The color of the carcass remained unaffected by storage, with mean values of L*=63.98, a*=1.54, and b*=14.21. Similarly, the color of the breast steak (BS) was not impacted by hyperbaric storage, with values of L*=55.67, a*=2.65, and b*=11.74. TBARS in all samples were below the limit of detection (0.17 mg MDA/kg). These findings suggest that although hyperbaric storage may potentially induce a prooxidant effect due to the high oxygen exposure, the applied conditions did not lead to lipid oxidation, probably due to the short period of the exposure [1].

IV. CONCLUSION

The use of hyperbaric storage had a very slight impact on *Campylobacter jejuni*. Both inoculated breast steak and neck skin exhibited a reduction in *Campylobacter* spp. of 0.55 log cfu/g, and 1.33 log cfu/g respectively. In non-inoculated samples hyperbaric storage resulted in 1 log cfu *Campylobacter* spp./g reduction on carcasses. Even so, this could be beneficial to increase safety level and accomplishing Commission Regulation (EU) 2017/1495. Yet, the effect of the tested hyperbaric storage conditions favored the growth of the spoilage microbiota, that might negatively impact the quality of the products. The applied conditions did not lead to changes in color or lipid oxidation. These preliminary findings indicate the need for exploring a new approach with different binomial conditions to optimize and effectively increase *Campylobacter* control while preserving and adequate the shelf-life of poultry products.

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EFFECTS OF TEMPERATURE AND TIME ON INDIGENOUS BACTERIA OF DRY-AGED BEEF

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I. INTRODUCTION

The dry aging process of beef exposes unpackaged meat to cold, facilitating surface drying while controlling temperature, relative humidity, and airflow [1]. It is a traditional method that not only influences its sensory characteristics but also the microbiological safety of the product. In this context, understanding the behavior of microorganisms such as *Escherichia coli* (*E. coli*), *Listeria sp*., and psychrotrophic bacteria (PCA), which could be part of the native microbiota of meat, plays a crucial role as indicators of food quality and safety. The presence of these microorganisms can indicate contamination and deterioration of the meat, impacting its safety and quality. Therefore, this study aims to evaluate the effect of different temperatures and periods of dry aging of beef on the growth of *Escherichia coli*, *Listeria sp.* and psychrotrophic bacteria.

II. MATERIALS AND METHODS

Six striploins (*Longissimus thoracis and lumborum*) weighing an average of 2.2 kg each, from Nelore bulls, were used in this study. The samples were taken from both the right and left sides of the carcasses. The samples were randomly distributed among three maturation chambers set at different temperatures: 0 °C, 3 °C, and 7 °C. The air velocity was 2.5 m/s, with a relative humidity of 75%. The samples were aged for 40 days, with evaluations conducted at 0, 10, 20, and 30-day intervals. To obtain representative samples from the internal portion of the cut, aseptic collection from five different points was performed. The enumeration of *Escherichia coli* was conducted on McConkey agar, with incubation at 37 °C for 24 hours. Enumeration of *Listeria sp.* was carried out on PALCAM agar, supplemented with selective antibiotic for *Listeria sp.*, and incubated at 37 °C for 48 hours. Enumeration of psychrotrophic bacteria was conducted on Plate Count Agar, incubated at 7 °C for 10 days. The results were expressed as log CFU/g. The data were analyzed using a mixed linear model including temperature and storage time as fixed effects, and replication as a random effect ($P < 0.05$), using Statgraphics® Centurion XVI version 16.1.11.

III. RESULTS AND DISCUSSION

The results of the bacterial counts are presented in Table 1. There was no interaction between storage time and temperature for the growth of *E. coli* in the internal portion of the meat ($P = 0.06$). However, significant differences were observed between storage days ($P < 0.001$), indicating a reduction in bacterial counts over time. Lower temperatures (0 °C and 3 °C) showed a trend toward lower bacterial counts, although this difference did not reach statistical significance.

Table 1. Microbiological counts in dry-aged beef under different temperature and time conditions.

Bacteria group	Temperature				
Day	0 °C	3 °C	7 °C	mean	*P value*

E. coli	0	5.72±0.34^{aA}	5.78±0.42^{aA}	5.68±0.43^{aA}	5.72±0.22^{a}	0.373
	10	1.80±0.11^{bA}	<1.69±0.00^{bA}	1.72±0.04^{bA}	1.74±0.04^{c}	0.500
	20	1.73±0.04^{bA}	<1.69±0.00^{bA}	2.12±0.28^{bA}	1.84±0.10^{c}	0.127
	30	<1.69±0.00^{bA}	1.80±0.08^{bA}	2.16±0.30^{bA}	1.88±0.11^{bc}	0.192
	40	1.94±0.09^{bB}	2.38±0.23^{bAB}	2.85±0.29^{bA}	2.39±0.15^{b}	0.030
	mean	2.58±0.26 ^{A}	2.68±0.27^{A}	2.91±0.26^{A}		
	P value	< 0.001	< 0.001	< 0.001		
Listeria sp.	0	5.05±0.22^{aA}	4.61±0.25^{aB}	4.81±0.22^{aAB}	4.83±0.13^{a}	0.001
	10	2.37±0.30^{bcA}	1.76±0.08^{cA}	2.33±0.36^{bA}	2.16±0.16^{c}	0.092
	20	1.88±0.11^{cB}	2.45±.34^{bcAB}	2.64±0.38^{bA}	2.32±0.18^{bc}	0.025
	30	<1.69±0.00^{cB}	1.79±0.10^{cB}	2.92±0.47^{bA}	2.13±0.19^{c}	0.001
	40	3.09±0.29^{bA}	2.99±0.31^{bA}	2.43±0.30^{bA}	2.84±0.18^{b}	0.157
	mean	2.82±0.21^{A}	2.72±0.20^{A}	3.03±0.21^{A}		
	P value	< 0.001	< 0.001	< 0.001		
Psychrotrophic bacteria	0	4.82±0.18^{abB}	5.82±0.22^{aA}	5.35±0.14^{bAB}	5.33±0.13^{a}	0.003
	10	5.69±0.38^{aA}	4.31±0.18^{bB}	4.07±0.11^{bcB}	4.64±0.21^{ab}	< 0.001
	20	4.23±0.14^{bB}	6.08±0.26^{aA}	3.58±0.30^{cB}	4.63±0.26^{b}	<0.0001
	30	5.39±0.11^{bB}	5.04±0.18^{bB}	6.84±0.20^{aA}	5.83±0.30^{a}	<0.0001
	40	3.18±0.10^{cB}	3.11±0.16^{cB}	5.54±0.69^{bA}	4.01±0.35^{c}	< 0.001
	mean	4.46±0.17^{B}	4.87±0.21^{AB}	5.00±0.24^{A}		
	P value	< 0.001	< 0.001	< 0.001		

Different uppercase letters in the same row indicate differences between temperatures ($P < 0.05$). Different lowercase letters in the column indicate differences between times ($P < 0.05$). Detection limit: 1.69 log CFU/g.

There was interaction between storage time and temperature for the growth of *Listeria sp.* ($P<0.05$). The count of *Listeria* sp. decreased over the storage time, regardless of temperature. Initially, the count varied from 4.61 to 5.05 log CFU/g and progressively decreased in all treatments. Over time, counts at 3 °C were lower than at 7 °C, with intermediate values at 0 °C. Van Damme et al. (2022) also found a stronger inhibitory effect on *L. monocytogenes* growth in beef aged for 42 days at 2 °C compared to 6 °C. The results for the count of psychrotrophs indicated a significant interaction (P < 0.001) between temperature and time. The psychrotrophic count exhibited lower levels at both 0 °C and 3 °C compared to 7 °C ($P<0.05$) throughout time. Interestingly, no significant difference in psychrotroph count was observed between the two lower temperatures (0 and 3 °C).

IV. CONCLUSION

Temperature and aging time influenced the microorganism count. The count of *E. coli* and *Listeria* showed a reduction over time in all treatments. Also, lower temperatures (0 and 3 °C) showed a trend for lower bacteria counts. As for psychrotrophic bacteria, 0 °C and 3 °C significantly inhibited the bacterial growth compared to 7 °C, highlighting a reduction in counts at temperatures ≤ 3 °C. These findings underscore the importance of lower temperatures and aging time to control the growth of the indigenous bacteria of beef during the dry aging process.

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Microorganisms isolated from fresh meats and meat products sold for consumption – detection of virulence and antimicrobial resistance genes

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I. INTRODUCTION

Meat and meat products are a significant part of the Portuguese diet. These foodstuffs have microorganisms responsible for the deterioration processes that reduce their shelf-life, representing economic losses and a considerable environmental impact. Also, they are a source of pathogens, with an important impact on public health, being a potential cause of foodborne diseases [1]. This study aimed to conduct a microbiological analysis of fresh meat and meat products from small local shops and hypermarkets. *S. aureus* and *L. monocytogenes* were isolated from the samples, to evaluate their phenotypic and genotypic antimicrobial resistance, the presence of virulence factors, and the ability to form biofilms.

II. MATERIALS AND METHODS

A collection of 75 samples of fresh meat preparations and meat-based products was undertaken, in northern Portugal, from local shops and hypermarkets. All samples were evaluated for mesophiles, Enterobacteriaceae, LAB (lactic acid bacteria), *Pseudomonas* spp., *L. monocytogenes*, *S. aureus,* and *E. coli*, according to the ISO norms. After isolating *L. monocytogenes* e *S. aureus* from samples, antimicrobial resistance was determined using the Kirby-Bauer disk diffusion method, against 14 e 12 antimicrobial agents, respectively. Resistance and virulence genes were evaluated with PCR.
Statistical analysis was performed with SPSS ® Statistics 29.0 for Windows, to determine if there were statistically significant differences in microorganisms' prevalence and counts.

III. RESULTS AND DISCUSSION

Total mesophilic counts presented the higher levels, followed by Enterobacteriaceae, LAB, *Pseudomonas* spp., *E. coli*, *S. aureus* and *L. monocytogenes* (Table 1). In general, the acceptability of samples was higher than 85%. Table 1 presents the microbiological counts (means and standard deviation) for type of product and microorganism evaluated.

Table 1- Microbiological counts (means and standard deviation) for type of product and microorganism evaluated.

Type of product	Total mesophilic counts (TMC)	Enterobacteriaceae	LAB	*Pseudomonas* spp.	*E. coli*	*S. aureus*	*L. monocytogenes*
Meat-based products	6.06 ± 1.44 ^{a}	1.84 ± 2.55 ^{b}	3.08 ± 2.37	0.22 ± 0.70 ^{c}	0.50 ± 1.07	nd	0.18 ± 0.41
Meatballs and hamburgers	6.19 ± 1.06 ^{a}	2.57 ± 1.63 ^{ab}	3.33 ± 1.95	2.82 ± 0.66 ^{a}	1.24 ± 0.91	0.35 ± 0.71	0.04 ± 0.13
Meat skewers	6.32 ± 1.13 ^{a}	2.67 ± 1.86 ^{ab}	3.14 ± 1.89	2.30 ± 1.75 ^{ab}	0.90 ± 1.08	0.28 ± 0.56	0.13 ± 0.39
Breaded meat	4.57 ± 1.30 ^{b}	1.86 ± 0.71 ^{ab}	2.64 ± 1.57	0.85 ± 0.98 ^{bc}	0.23 ± 0.41	nd	0.20 ± 0.22
Minced meat	6.01 ± 0.73 ^{a}	3.79 ± 1.27 ^{a}	4.14 ± 0.96	1.35 ± 1.31 ^{b}	0.52 ± 0.81	0.21 ± 0.66	0.07 ± 0.29
Fresh sausage	5.08 ± 0.85 ^{ab}	2.03 ± 0.37 ^{ab}	3.36 ± 0.77	0.60 ± 0.73 ^{bc}	0.13 ± 0.21	nd	nd
Sig.	*	**	ns	***	ns	ns	ns

Sig. – level of significance; ns – not significant (p≥ 0.05); *significant (p< 0.05); **very significant (p< 0.01); *** highly significant (p< 0.001)
For each type of product, means that do not have the same letter, differ significantly (p < 0.05). nd – not detected

The prevalence of *S. aureus* and *L. monocytogenes* was 10.67% and 17.33%, respectively. *S. aureus* isolates were resistant to penicillin (52.6%), tetracycline (44.4%), chloramphenicol (36.8%) and tobramycin (26.3%). Virulence genes found were: *tet*K (31.58%), cat_{pc223} (21.05%) e *bla*Z e *ant*(a')-Ia (15.79%). *L. monocytogenes* isolates were resistant to trimethoprim-sulfamethoxazole (85.71%), ciprofloxacin (38.10%), meropenem (33.33%), tetracycline and erythromycin (28.57%), rifampicin (23.81%) and kanamycin (14.29%). Virulence genes found were: *tet*K (23.81%), *aad*A, *tet*L, and *bla*OXA-48 (14.29%), *erm*C and *msr*A/B (4.76%). Four *S. aureus* isolates and six *L. monocytogenes* isolates exhibited a multi-resistance profile. All isolates were able to form biofilms at 24h and 48h, and there were some highly productive biofilm strains.

IV. CONCLUSION

This study is of great relevance as it provides us with a better understanding of the microbiological quality of meat and, consequently, of good hygiene practices from animal slaughter to retail establishments. It is important to understand the characteristics of the bacterial strains, especially because these foodstuffs are a reservoir of pathogenic and spoilage bacteria that can cause food poisoning.

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INFLUENCE OF EMERGING PRESERVATION TECHNOLOGIES ON THE SPOILAGE PATTERN OF A COOKED MEAT AND BREAD-BASED SAUSAGE

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I. INTRODUCTION

The spoilage of meat products is a complex multifactorial phenomenon that involves the activity of the spoilage microbiota, chemical and physical modifications, particularly lipid oxidation, and the interaction between them [1]. *Alheira* is a meat product made with shredded cooked pork, chicken meat, and wheat bread moistened in the meat cooking broth. After filling, it is slightly smoked and dried. [2]. The cooking of meat significantly reduces the microbiota. However, it is further manipulated after the cooking, resulting in a product with a diversified microbiota that includes high lactic acid bacteria (LAB) counts, which have a paradigmatic effect once it contributes to product acidification, which is usually well accepted when limited. Among the technologies available to reduce spoilage microbiota, encapsulated bacteriocins and high hydrostatic pressure (HHP) have been successfully applied to meat products. Due to its selective effect on the microbiota, the result might be a dysregulation of the microbial ecosystem with unpredictable consequences. We aimed to understand the effect of encapsulated bacteriocin and its combination with HPP on the sensorially detected spoilage pattern of Alheira.

II. MATERIALS AND METHODS

Three independent batches of alheiras were produced, as described by Borges et al. [3], and vacuum vacuum-packaged. We designed the experiment with three factors: one control, one supplemented with an encapsulated bacteriocin [4], and another with the encapsulated bacteriocin and further submitted to an HPP treatment (600 MPa, 960 s; N.C. Hyperbaric, Wave 6,000/135; Spain). The *alheiras* were stored at 5°C for four months. Freshly prepared and at the end of each month of storage, the samples were withdrawn and frozen to use a reverse design for sensory analysis. A consumer group was recruited (n=80, 33% men, aged from 18 to 69 years old) to perform a Rate All That Apply (RATA). The vocabulary included to describe the spoilage was fermented, alcohol, rancid, spoiled and putrid to evaluate aroma, acid taste, and fermented and rancid for the flavour dimension. A yes/no question asking about the intention to consume was used. For each characteristic, the consumers were asked to indicate if it was present and, if it was, to rate its intensity on a five-point scale [5]. Binary logistic regression was used to evaluate, for each treatment, which characteristics influence the consumption intention based on freshness (XlStat, Addinsoft, Paris).

III. RESULTS AND DISCUSSION

The profile of sensory deterioration (table 1) of control alheiras is characterised by the spoiled aroma and acid taste, which reduces consumption intention (OR=0.544; OR=0.749, respectively). When we use encapsulated bacteriocins, the two sensory attributes, spoiled aroma and acid taste, also had a significative impact on the reduction of the consumption intention, with reduction dimensions similar to

the control. In this group of samples, the acidic aroma was a favourable sensory trait, increasing consumption intention (OR=1.217). Similarly, when the alheiras treated with encapsulated bacteriocins were HPP treated, a similar sensory trait emerged as favourable to the consumption intention, the fermented aroma (OR=1.346). The rancid aroma only contributed to the lower consumption intention in samples simultaneously treated with encapsulated bacteriocin and HPP.

Table 1 Logistic regression model for the spoilage sensory attributes of alheira (control and HHP-treated).

Variables	β	SE	p	Odds ratio	95% CI
Control					
Spoiled aroma	-0.609	0.292	0.037	0.544	0.307-0.964
Acid taste	-0.289	0.121	0.006	0.749	0.592-0.949
Encapsulated bacteriocin					
Acidic aroma	0.196	0.089	0.027	1.217	1.023-1.448
Spoiled aroma	-0.913	0.335	0.006	0.401	0.208-0.773
Acid taste	-0.302	0.123	0.014	0.739	0.581-0.941
Encapsulated bacteriocin + HHP					
Fermented aroma	0.297	0.151	0.050	1.346	1.001-1.812
Rancid aroma	-0.439	0.209	0.036	0.645	0.428-0.972

IV. ICONCLUSION

The use of encapsulated bacteriocins to control the spoilage microbiota slightly affected the sensory spoilage of alheira. Still, the two main determinants of the reduction of acceptability – the spoiled aroma and acid taste had a similar trend. Acidic and fermented aroma had a positive effect on encapsulated- and HPP-treated alheiras. These results stress the importance of understanding spoilage processes, which could result in different sensory traits that could be favourable for the consumers' acceptance of the product.

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USE OF THE MICROBIAL GROWTH PREDICTOR TO INDICATE CHANGES IN THE SAUSAGE'S APPEARANCE AFFECTING THE SALE FLUX IN MARKETS

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I. INTRODUCTION

The growth of ropy slime producing bacteria is a common defect impairing superficial color of vacuum-packaged meat products. Thus, the visual aspect created by the formation of ropy slime impacts the consumer appraisal and may cause the product rejection before the expiry date prescribed by the manufacturer [1]. The food industry requires a relatively short time to obtain the information needed to determine the shelf-life of food products. For practical reasons, when the actual product shelf-life is long, the industry usually uses accelerated tests that considerably shorten the time spent to obtain relevant experimental data [2]. The microbial growth predictor, named *MicroLab_ShelfLife*, was developed to perform a durability study by an *in vitro* and an *in silico* trial, under a dynamic temperature profile, and preserving the intrinsic features of the products. The entrance of the natural microbiota of vacuum-packaged cooked sausage into the stationary phase is a suitable borderline to indicate the retention of the original attribute related to the product appearance [3]. This study aimed to evaluate the reliability of the microbial growth predictor to indicate when most consumers will refuse the product in markets.

II. MATERIALS AND METHODS

Vacuum-packaged cooked sausages were manufactured by two different meat industries, located in the states of Rio de Janeiro (A) and Minas Gerais (B), Brazil. A durability study was performed according to the *MicroLab_ShelfLife* protocol. In brief, the sample group was composed by five packages. One package was analysed soon after being received in the laboratory to count the initial microbial load (time zero). Natural microbiota was stimulated to grow by pair incubation of the packages at lower (7 °C) and higher (36 °C) temperatures. The method ISO 4833-1 (2013) was used to enumerate the microorganisms in samples [4]. Results related to the colony counting were entered in the computational predictive modelling package to obtain the microbial growth curve at a dynamic temperature profile. An electronic device (QII343, XpressPDF Logger, Emerson, USA) was used to elucidate the temperatures to which the products were exposed during sale in the market. The results were compared with the data reported by the seller corresponding to the decline of more than 50 % in the normal sale flux in the market.

III. RESULTS AND DISCUSSION

The microbial growth predictor estimated retention of the original properties of the products until 28 (A) and 57 days (B). In market, a decline of more than 50 % on the normal sale flux was observed after 29 (A) and 55 days (B) (Figure 1).

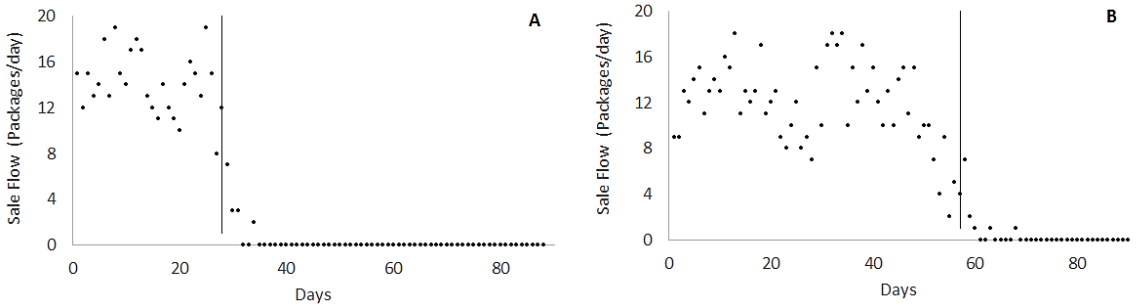

Figure 1. Daily sale flow in the market. Vacuum-packaged cooked sausages were produced in the states of Rio de Janeiro (A) and Minas Gerais (B), Brazil.

The establishment of criteria linking microbial growth to alterations in product appearance remains subject to ongoing debate [5]. While consumer acceptance or rejection of sausages in markets varies based on individual assessments, establishing a threshold aligned with the entry of natural microbiota into the stationary phase appears promising for indicating changes in the original properties, particularly the superficial color, of vacuum-packaged cooked sausages.

IV. CONCLUSION

The *MicroLab_ShelfLife* microbial growth predictor demonstrated robustness and accuracy in detecting alterations in the inherent properties, particularly the superficial color, of vacuum-packaged cooked sausages. Its efficacy suggests it can serve as a valuable tool for signalling when the product may reach a state unacceptable to most consumers in the market.

ACKNOWLEDGEMENTS

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USE OF BIOPRESERVATIVE TO ENSURE MICROBIOLOGICAL SAFETY IN BACON

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I. INTRODUCTION

The Portaria SDA nº 748, enacted by the Brazilian government on February 8, 2023 [1], regulated the identity and quality standards for bacon. It stipulates that for bacon intended for storage at room temperature, the permissible maximum water activity (*aw*) level is set at 0.85. This standard is grounded in the understanding of the growth dynamics of *Staphylococcus aureus*, a bacterium notorious for its potential to produce harmful enterotoxins. However, adherence to this *aw* value impacts the physicochemical composition and sensory attributes of bacon produced in Brazil. Consequently, there arises a necessity to explore alternative methodologies that can effectively mitigate the growth of *S. aureus* without compromising the prescribed *aw* levels and the requisite storage temperatures for retail distribution. The objective of this study is to devise a novel treatment approach, involving the indirect incorporation of a biopreservative produced in an axenic fermentation system with *Lacticaseibacillus paracasei*, to inhibit the growth of *S. aureus* in bacon.

II. MATERIALS AND METHODS

Bacon samples were manufactured at BRC Ingredientes Ltda, located in the city of Rio Claro, state of São Paulo, Brazil. Three distinct processing methods were performed to obtain bacon with various *aw* levels. The brine formulation of each treatment is showed in Table 1.

Table 1. Brine formulation for producing bacon with various water activity level.

Ingredients	Dry Salting	Immersion Salting	Injection Saltin
Water/ice		74.0 %	74.0 %
Bacon blend[a]	20.0 %	5.0 %	5.0 %
Sodium chloride	34.0 %	15.0 %	15.0 %
Curing salt[b]	3.5 %	1.0 %	1.0 %
Sucrose	20.0 %	5.0 %	5.0 %

[a]52.1 % of sodium chloride, 34 % of sodium tripolyphosphate, 12 % of sodium erythorbate, and 1.9 % of sucrose.
[b]90 % of sodium chloride, 6 % of sodium nitrite, and 4 % of sodium nitrate.

To obtain a sample group B1, with an *aw* value approximating 0.85 using the dry salting method, the powder mixture was blended onto pork belly at a ratio of 1:6, followed by cold storage at 7ºC for 6 days. For sample group B2, aiming for an *aw* value close to 0.92 through the immersion salting method, pork bellies underwent complete immersion in brine for 2 days at 7°C, followed by an additional 3 days of cold storage at 7°C. Lastly, to produce sample group B3, with an *aw* value exceeding 0.95, pork bellies were injected with a 20.6 % of brine solution and then cold-stored at 7°C for 24 hours. The pieces were subsequently cooked in an oven at 70°C for 40 minutes, 75°C for 40 minutes, and a final 4-hour period at 80°C. Each sample group was subdivided into three subgroups: a blank group without treatment (B), a control group with 1.0 % of sterile water (C), and a treatment group with 1.0 % of biopreservative (T). Both water and biopreservative were introduced into the packaging to hurdle microbial growth at the product-package interface. The challenge test was performed according to the protocol of the method *MicroLab_ShelfLife* at various temperature profiles [2]. The upper limit of 6.0 log cfu/g was considered to indicate the end of the shelf-life.

III. RESULTS AND DISCUSSION

The *aw* values of the bacon produced by dry, immersion and injection brine salting methods were 0.859, 0.938, and 0.968, respectively. The results of the challenge test are shown in Table 1.

Table 1 – Duration of bacon produced with various water activity.

			Treatments								
	Incubation conditions		Dry salting			Immersion salting			Injection salting		
	Temperature (°C)	Time (dias)	B1-B	B1-C	B1-T	B2-B	B2-C	B2-T	B3-B	B3-C	B3-T
in-vitro trial (Log cfu/g)	7	0	3,71	3,88	3,79	3,66	3,76	3,81	3,66	3,76	3,81
		4	3,89	3,99	3,80	3,95	4,01	3,81	3,95	4,01	3,80
		6	4,37	4,45	3,82	4,42	4,14	3,81	4,42	4,14	3,79
	36	4	4,70	4,80	< 2,60	4,59	4,66	3,80	4,59	4,66	3,82
		6	6,49	6,61	< 2,60	6,42	5,12	3,80	6,42	5,12	3,85
		Storage temperature profile									
*Ngrowth - (Log cfu/g/day)**		*Cold a 7 ºC*	0,2982	0,2771	-0,0100	0,3064	0,2078	0,0000	0,4839	0,8433	0,0001
		Cold with abuse	0,3429	0,3214	-0,0150	0,3473	0,2360	0,0000	0,5839	0,9493	0,0002
		Room at 25ºC	0,4800	0,4572	0,0002	0,4724	0,3224	0,0000	0,6574	1,4323	0,0000
*Ndeceleration - (Log cfu/g/day)***		*Cold a 7 ºC*	0,0983	0,0888	-0,0050	0,1057	0,0712	0,0000	0,2833	0,4432	0,0000
		Cold with abuse	0,1375	0,1310	0,0001	0,1354	0,0924	0,0000	0,2847	0,5943	0,0000
		Room at 25ºC	0,1223	0,1146	-0,0060	0,1239	0,0842	0,0000	0,2483	0,6432	0,0000
*Durability (days)****		*Cold a 7 ºC*	21	12	*indefined*	22	11	*indefined*	20	10	745
		Cold with abuse	18	10	*indefined*	18	10	*indefined*	17	10	619
		Room at 25ºC	15	7	*indefined*	1	7	*indefined*	14	7	245

* Daily growth of the microbial population (log cfu/g) in the exponential growth phase. ** Daily microbial population growth (log cfu/g) in the deceleration phase. *** An upper limit of 6.0 cfu/g of *S. aureus* was considered to indicate the end of the durability.

The findings suggest that solely reducing *aw* levels to 0.85 might not adequately impede the growth of *S. aureus* on bacon surfaces. This concern is exacerbated with retention of water within the packaging, as showed in group control. Conversely, promising outcomes were observed by indirect application of the biopreservative to inhibit the growth of *S. aureus* at the product-packaging interface.

CONCLUSION

The application of the biopreservative at the interface between the product and the packaging demonstrated superior efficacy in inhibiting the growth of *S. aureus* compared to the reduction of *aw* values.

ACKNOWLEDGEMENTS

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Antimicrobial Properties of Sodium Alginate Films Loaded with Laurel and Olive Leaves Extracts for Extending the Shelf-life of Fresh Meat

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I. INTRODUCTION

The application of edible films and coatings has emerged as one of the most promising approaches to achieve the challenging and important relationship between preservation efficacy and environmental responsibility. This study aimed to perform *in vitro* studies to investigate the effectiveness of alginate films impregnated with laurel and olive leaf extracts for extending the freshness and quality of meat.

II. MATERIALS AND METHODS

Leaves of laurel and olive were harvested, washed, dried at 25ºC under air circulation until constant weight and milled in a 1 mm sieve. Extractions were carried out by ultrasonic-assisted extraction technique in an ultrasound bath. Twenty grams of dried milled leaves were mixed with 100 mL of 70:30 (v/v) ethanol:water solution, sealed in an Erlenmeyer flask and placed into the bath with 3 L of destilled water at 25ºC ±5ºC for 1h. The mixture was centrifuged at 5000 × g for 10 min. After centrifugation, the solvent was removed in a rotary evaporator at 38 ºC, under vacuum, and freeze-dried.
The films were obtained by mixing 1% (w/v) of sodium alginate (SA) and 0.5 % (w/v) of glycerol in distilled water under agitation overnight. Then, laurel leaves extract (LLE) and olive leaves extract (OLE) were dissolved in distilled water, stirred for 1 h, filtered under vacuum, and added to the film-forming solutions at 1:1 and a final concentration of 1 and 2%. All solutions were stirred for 1 h, homogenized with an Ultra-Turrax at 10000 rpm for 2 minutes, and degassed under vacuum. The film-forming solutions were cast in polystyrene petri plates, dried at 35 ºC (air circulation) for 24 h, and conditioned in desiccators containing a saturated solution of $Mg(NO_3)_2.6H_2O$ at 53% of relative humidity and 20 ºC before analysis. Antimicrobial activity for a maximum of 2% extract was conducted following the broth microdilution method using an ELISA plate reader at 600 nm against *Listeria monocytogenes* ATCC 7973, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 14028, *Enterococcus faecalis* ATCC 19433 and *Escherichia coli* ATCC 11775 at ~$5x10^5$ CFU/mL. The antimicrobial activity of films was determined by the viable cell count assay method according to Nouri et al. (1) with slight modifications. Samples with 0.1 g were immersed in 2 mL of brain heart infusion broth (BHI) inoculated with ~10^6 CFU/mL of the microorganisms previously mentioned. Samples were incubated at 37 ºC and counts were obtained at 0 and 24h. Microorganisms concentrations were standardized by OD600. All analyses were performed in duplicate.

III. RESULTS AND DISCUSSION

When OLE was combined with LLE (1:1), the minimal inhibitory concentration MIC value was achieved at 1% for *S. aureus* and 2% for *L. monocytogenes*. For other microorganisms, MIC was not achieved, which indicated that it is higher than 2%. Despite this, it was possible to observe that the absorbance decreased for higher extract concentrations. The minimum bactericidal concentration (MBC) was achieved at 2% for *S. aureus*.

Table 1 – Antimicrobial activity of alginate films impregnated with LLE+OLE extracts: counts (mean, log UFC/g) at 0 h and after 24 h of incubation.

Sample	*S. aureus*	*L. monocytogenes*	*E. faecalis*	*S.* Typhimurium	*E. coli*
CNT 0h	7.26±0.06^{b}	7.32±0.03^{d}	7.19±0.05^{c}	7.09±0.04^{c}	7.25±0.08^{c}
CNT 24h	10.66±0.24^{a}	10.48±0.19^{a}	10.17±0.09^{a}	10.68±0.05^{a}	12.05±0.02^{a}
SA	10.70±0.06^{a}	9.93±0.13^{ab}	10.02±0.14^{a}	10.77±0.03^{a}	12.05±0.03^{a}
SA+LLE0.5%+OLE0.5%	6.00±0.26^{c}	9.55±0.27^{bc}	9.65±0.02^{ab}	9.51±0.06^{b}	11.45±0.11^{b}
SA+LLE1%+OLE1%	5.31±0.05^{d}	8.84±0.19^{c}	9.03±0.33^{b}	9.35±0.04^{b}	11.20±0.05^{b}
P	<0.001	<0.001	<0.001	<0.001	<0.001

CNT – control; SA – sodium alginate; LLE – laurel leaves extract; OLE – olive leaves extract; Means with different letters (columns) differ significantly, P <0.05.

No antimicrobial activity was observed for the alginate-based film (SA) without extracts compared to the control (CNT 24h). With the addition of extracts, lower counts were observed at 24 h compared to CNT 24h, mainly for Gram-positive microorganisms.
After 24 h, and compared to CNT at 0 h, the best results were observed for SA + LLE 1% + OLE 1% against *S. aureus* with a reduction of 1.95 log CFU/g which demonstrates a good antimicrobial activity, although not enough to consider the compound bactericidal (≥ 3 log10 reduction). SA+LLE0.5%+OLE 0.5% also demonstrated good results against *S. aureus* with a reduction of 1.32 CFU/g. The antimicrobial effect observed for the other microorganisms was less desirable. However, a reduction in counts was observed after 24 hours compared to CNT24h. Notably, lower counts were obtained for *L. monocytogenes*, with a difference of 1.64 log CFU/g compared to CNT24h (P<0.001).

IV. CONCLUSION

The incorporation of plant-derived extracts demonstrated encouraging outcomes in reducing microbial counts within 24 hours, with a particular focus on Gram-positive microorganisms. The observed antimicrobial efficacy was notably pronounced against *S. aureus*. Future investigations could explore the potential of fine-tuning the concentrations or synergistic combinations of these extracts to enhance their antimicrobial potency, thereby advancing their application in meat preservation methods.

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Identifying features of abscess caused by foot-and-mouth disease (FMD) vaccination in pork meat and detection through hyperspectral imaging

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I. INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral illness that affects cloven-hoofed animals such as cattle, pigs, and goats, leading to substantial economic losses. Mass vaccination is a crucial preventative measure; however, vaccination can result in pork abscesses, compromising quality and causing financial setbacks. In Korea, vaccination is compulsory following FMD outbreaks, yet this has led to the emergence of abscesses in certain pork products. In this study, we delve into hyperspectral imaging techniques aimed at identifying FMD vaccine-associated abscesses, thereby offering potential for objective assessment and enhancing vaccination efficacy.

II. MATERIALS AND METHODS

The samples used in this study were pork hams obtained from a local meat factory. Pork meat samples with abscesses were collected from slaughtered pork carcasses, transported in a low-temperature vacuum, and stored in a refrigerator at 5°C until just before the experiment. Hyperspectral image acquisition was conducted using three different hyperspectral imaging systems (1000-2500 nm short infrared (SWIR), 400-1000 nm Vis/NIR, and 400-800 nm fluorescence hyperspectral imaging systems). After obtaining the hyperspectral images, the pork samples were used as reference data for physicochemical experiments (proximate contents, microbiome analysis, and H&E staining). The acquired image data were used for intensity calibration, and the mean spectrum was extracted from the meat, fat, and abscess areas. Finally, the total spectrum was calculated with 300 data points from the meat, 300 from the fat, and 300 from the abscess area for a classification model using partial least squares discriminant analysis (PLS-DA). Classification models were created using each set of spectral data. An ANOVA test was conducted to assess significance, and after identifying significant parameters, Duncan's multiple range test was performed for further analysis(P<0.05).

III. RESULTS AND DISCUSSION

Table 1 presents the optimal results for each hyperspectral classification model. The best classification model for Vis/NIR achieved a 97.0% accuracy rate in abscess detection. The mean normalization preprocessing method proved the most effective for data preprocessing. Validation results from the fluorescent hyperspectral imaging system demonstrated a 96.2% accuracy rate using the mean normalization preprocessing method. Additionally, the SWIR imaging system yielded a 98.9% accuracy rate with the MSC preprocessing method. Latent variables (LV) are crucial in explaining model complexity, as high LV values can lead to overfitting (Kong et al., 2022). Therefore, it is essential to consider LV values when selecting the appropriate classification model. The SWIR classification

model exhibited lower LV values and higher classification accuracy percentages in this study. This suggests that the SWIR hyperspectral system can efficiently detect -OH, -CH, and -NH bonding overtones compared to other ranges (Kim et al., 2023), leading to enhanced detection accuracy. Furthermore, the SWIR range appears capable of detecting collagen areas within fibrotic muscle tissue, although this data is not presented in this abstract. Consequently, the SWIR hyperspectral camera system or SWIR spectrum range holds promise for practical applications in abscess detection within real-world settings.

Table 1. Accuracy of pork abscess detection algorithm for each hyperspectral camera system using different preprocessing methods.

System type	Preprocessing method	LV	Calibration Set (630 spectrums)				Validation Set (270 spectrums)			
			ABS (%)	Meat (%)	Fat (%)	Total (%)	ABS (%)	Meat (%)	Fat (%)	Total (%)
Vis/NIR hyperspectral system	Mean norm	9	96.2	100	99.1	98.4	96.7	100	94.4	97.0
	Raw spectrum	21	45.2	95.7	44.1	61.7	42.2	100	49.4	63.9
Fluorescent hyperspectral system	Mean norm	11	94.6	100	100	98.2	88.7	100	100	96.2
	Raw spectrum	17	47.3	95.8	51.2	64.7	44.3	96.6	57.6	66.2
SWIR hyperspectral system	MSC	5	98.1	99.5	99.1	98.9	97.8	100	98.8	98.9
	Raw spectrum	11	24.8	99.5	40.8	55.0	15.6	90.8	35.4	47.2

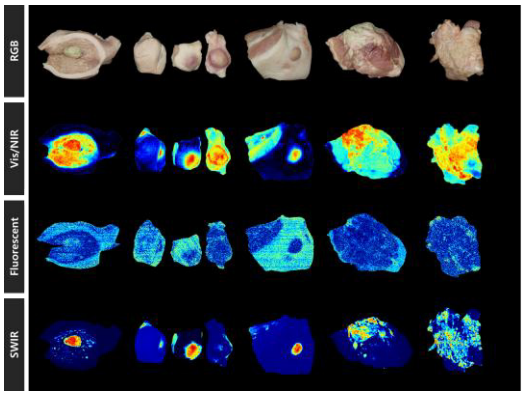

Figure 1. Chemical images from SWIR hyperspectral camera model for pork abscess detection.

IV. CONCLUSION

This study investigated the potential for detecting FMD vaccine-induced abscesses in pork using Vis/NIR, fluorescence, and SWIR hyperspectral imaging and confirmed the characteristics of abscesses. Vis/NIR and SWIR imaging led to high classification accuracy. However, fluorescence images showed lower accuracy, with the SWIR region's hyperspectral system exhibiting the highest accuracy in sample classification. Therefore, it is deemed advantageous to utilize the wavelength range of the SWIR region for device construction in the future.

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Control of *Staphylococcus aureus* During Biltong Production

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I. INTRODUCTION

Biltong is a dried meat product native to South Africa. Unlike jerky, biltong is dried at ambient temperatures and relies on salt concentration and acidity to inactivate pathogens. Throughout history, different cultures, countries, and methods have been used to dry meat, many of which are still used today [1]. Drying meat removes water which prevents enzymes and bacteria from reacting with the food [2]. Salt is commonly used to preserve meat as it reduces water activity and contributes flavor [3]. Biltong is made from strips of meat, typically beef. It can be dipped in spices and acidic liquids like vinegar, before drying to add flavor but to also provide antimicrobial activity [3]. Traditional South African dried biltong is prepared by hanging the strips of meat outside for one to two weeks [3] but is made commercially under more controlled conditions.

Staphylococcus aureus is commonly found in foods exposed to ambient to higher temperatures along with frequent handling, and it is associated with dried meat products due to its tolerance for low water activity (a_w) and high salt concentrations. With the increasing popularity of biltong worldwide [3], it is critical to understand potential microbial risk, and how it may be managed. In the United States, *S. aureus* guidelines for final ready-to-eat meat products state that no more than 2 logs of growth should occur during processing, which includes drying [5]. Due to an acidic marinade and dip, it is essential to consider if acid stress adaption may potentially increase resiliency to treatment. The objective of this study is to 1) evaluate the effectiveness of acid marination and drying of biltong to control *S. aureus*, and 2) determine if prior adaptation to acid stress increased resiliency in *S. aureus*.

II. MATERIALS AND METHODS

Biltong strips (100 ± 5.0 g; 2 cm x 6 cm x 8 cm) were cut from beef round. Stationary phase *S. aureus* strains (ATCC12600, 13565, and 25923) were used as the inoculum. Each trial contained two pathogen groups, acid stressed *S. aureus* (grown in tryptic soy broth (TSB) with 1% glucose solution) and unstressed *S. aureus* (grown in TSB only). Each biltong strip was inoculated to a target concentration of 8 log cfu/g. After 30 min attachment, each strip was dipped in a 5% lactic acid solution for 5 seconds. Samples were plated before lactic acid dip and immediately after lactic acid dip. After overnight refrigeration, strips were added to a marinade solution of vinegar (2% v/w), salt (2% w/w), black pepper (1% w/w), brown sugar (1.5% w/w), and coriander (1% w/w) and vacuum sealed. Strips were marinated for 24 h at 4°C, then neutralized with 1% w/w sodium bicarbonate for 10 min. Strips were then hung in a Biltong box maintained at 27°C and 55% humidity. From each pathogen group, two strips were taken for enumeration each day for 7 d. Serial dilutions were mixed with molten TSA and overlayed onto Baird Parker agar. Plates were incubated for 20-25 h at 35°C and enumerated. Water activity was measured daily on non-inoculated strips using a water activity meter (Aqua Lab CX-2, Decagon Devices, Inc., Pullman, WA, USA). Microbial inactivation data was fitted with reparametrized Weibull inactivation models using the gnls function in the nlme package in R 4.3.1.

III. RESULTS AND DISCUSSION

Combined lactic acid dip, marination, and 7 d of drying resulted in 3.5±0.24 log cfu/g reduction ($P<0.0001$) with unstressed *S. aureus.* While reductions were less than the recommended 5 log reduction for *Salmonella*, any reduction is well below processing RTE guidelines for *S. aureus* (<2 logs of growth). A

concern is that acid-adapted *S. aureus* may be more resilient to the biltong process [4]. However, in this study, stress adapted *S. aureus* were much less resilient to treatment (5.1±0.25 log cfu/g reduction, P<0.001) compared to non acid stressed *S. aureus*.

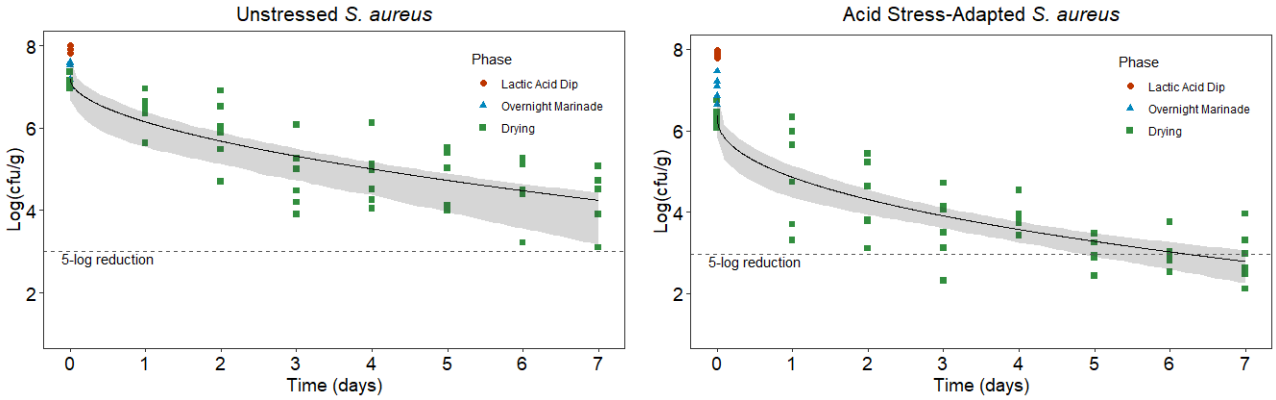

Figure 1. *Inactivation of Staphylococcus aureus* after lactic acid dip, acid marination, and 7d drying. Unstressed *S aureus* were 18h stationary phase cultures, whereas acid stress adapted included 1% glucose in the grow up to lower the pH from 7.0 to 4.0. Black line indicates best fit by Weibull model. Shaded area indicates 95% confidence interval.

IV. CONCLUSION

Risk of *S. aureus* growth and toxin production during biltong manufacture is very unlikely. Acid adaption did not impart any additional resilience compared to stationary phase inoculum suggesting stationary phase cultures are adequate to assess *S. aureus* control in acid-marinated beef products. Future studies should assess the taste of biltong without the neutralization step after marination. Researchers should also explore microbial lethality without sodium bicarbonate neutralization to evaluate potential improvements in food safety.

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USE OF MESQUITE PROPOLIS EXTRACT AS A NATURAL ADDITIVE FOR PRESERVING MINCED PORK MEAT

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I. INTRODUCTION

Lipid oxidation and microbial growth are risk factors associated with the deterioration of pork quality and negatively impact consumer purchase intentions. Therefore, the use of additives of synthetic origin is one of the alternatives that the meat industry follows to reduce both problems [1]. However, the uncontrolled use of synthetic additives poses health risks, a reason for concern among consumers that are increasingly reluctant to the consumption of meat products formulated with such food preservatives [1,2]. In this context, honeybee products are considered an important source of chemical compounds with bioactive properties, including polyphenols, which could be considered as novel food additives [3]. The aim of this study was to investigate the potential use of mesquite propolis extract (MPE) as a natural antioxidant and antimicrobial additive for preserving minced pork meat.

II. MATERIALS AND METHODS

Polyphenols from propolis, collected from two apiaries from Pueblo de Álamos (México), were extracted with water (1:10) by maceration-assisted extraction (150 rpm/25 °C/24 h). The solution was filtered (Whatman No 4-filter paper), dried using a freeze drier, and the obtained mesquite propolis extracts (MPE) were subjected to evaluation for total polyphenols contents (phenolic, TPC; flavonoids, TFC) and free-radical scavenging and reducing power activities (DPPH and FRAP, respectively). Also, the antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* were evaluated. Butylhydroxytoluene and gentamicin were used as positive controls. Minced pork meat (*M. semimembranosus*, 24 h *postmortem*; 1.5% salt; 10% fat) was assigned to four treatments (Control, without antioxidant; MPE #1 and #2, extracts from apiaries #1 and #2, both at 500 ppm; T3, butylhydroxytoluene toluene-BHT at 500 ppm), cooked in a water bath (65 °C for 60 min), and subjected to pH, thiobarbituric acid reactive substances (TBARS), and total bacteria counts (TBC) tests [4,5]. Obtained data (n=6) were subjected to ANOVA and Tukey-Kramer's multiple comparison tests at $P<0.05$ (NCSS v11).

III. RESULTS AND DISCUSSION

The results depicted in Table 1 showed that MPE #2 showed the highest TPC and TFC values ($P<0.05$). With respect to antioxidant activity, no significant differences were found between natural and synthetic antioxidants for DPPH values ($P>0.05$), while MPE #2 and BHT showed the highest FRAP values ($P<0.05$). Concerning antimicrobial activity, the gentamicin exerted the highest antimicrobial activity, and the main antibacterial effect observed for all treatments was against *S. aureus* compared to *E. coli* ($P<0.05$). As shown in Figure 1, MPE #1 and MPE #2 reached higher pH values respect to control ($P<0.05$). The lowest TBARS values ($P>0.05$) were obtained in MPE #2 samples, while both MPE #1 and MPE #2 samples showed lower TBC values as compared to other treatments ($P<0.05$).

Table 1 – Polyphenols content and bioactivity of MPE.

Traits	Polyphenols		Antioxidant activity		Antimicrobial activity	
	TPC	TFC	DPPH	FRAP	*S. aureus*	*E. coli*
MPE #1	174.86±0.66^{a}	30.69±3.48^{a}	89.60±0.02^{a}	1.02±0.04^{a}	0.21±0.01^{b}	0.33±0.01^{b}
MPE #2	286.81±2.16^{b}	70.99±1.86^{b}	89.07±0.05^{a}	1.39±0.06^{b}	0.19±0.01^{b}	0.33±0.01^{b}
Std.	-	-	91.04±0.06^{a}	1.41±0.01^{b}	0.10±0.01^{a}	0.12±0.01^{a}

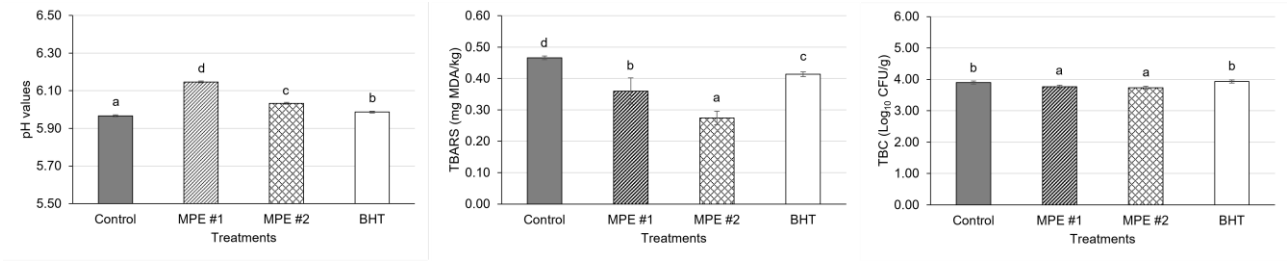

Figure 1. Effect of treatment and cooking period on pH, TBARS and TBC values of pork meat.

Propolis is a resinous material that bees process using the plant material surrounding the apiary. This honey coproduct has antioxidant and antimicrobial properties associated to its polyphenols composition. Therefore, it has been proposed as a natural food additive [1,5]. In agreement with our results, the inclusion of propolis extracts (1.5, 2.0, and 5.0%) in raw ground beef has enhanced the oxidative status of minced beef by stabilizing pH and TBARS values during storage (7 °C/9 days), with a concomitant reduction in TBC values [6].

IV. CONCLUSION

MPE is an alternative source of antioxidant and antimicrobial compounds that can be used as a natural additive in the meat industry.

ACKNOWLEDGEMENTS

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PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF MINCED PORK TREATED WITH MESQUITE HONEY

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I. INTRODUCTION

Mexico is the fifth largest honey exporter worldwide and the beekeeping industry has significant social and economic implications with approximately 43,000 beekeepers that produce around 61,000 tons of honey annually [1]. Honey has been valued for centuries not only for its sensory attributes like flavor, but also for its nutraceutical properties [2]. Honey exhibits a remarkable ability to neutralize free-radicals responsible for oxidative stress in the human body. This antioxidant potential of honey makes it an attractive option to treat diseases related to oxidative stress, such as cancer and heart disease [2,3]. In addition, honey contains antimicrobial and antioxidant compounds that can offer effective protection against oxidative deterioration of foods, prolonging their shelf-life without compromising food quality and safety [3]. However, it has been reported that the floral origin plays a very important role in the chemical composition and biological properties of honey [4]. The aim of this study was to investigate the potential use of mesquite honey as an antioxidant and antimicrobial additive for minced pork meat.

II. MATERIALS AND METHODS

Honey samples (2.5 L each) derived from blossoms of the Velvet mesquite (from *Prosopis velutina*) were collected from two apiaries located at two municipalities (Pueblo de Álamos and Carbó) of the Sonoran Desert, Northwestern México. Upon arrival to the laboratory, honey samples were diluted with water (1:10) at 10,000 rpm/25 °C/1 min. The resultant solutions (MH1 and MH2) were analyzed for total polyphenols contents (phenolics, TPHC; flavonoids, TFC; tannins, TTC), Free-Radical Scavenging (FRSA) and reducing power activities (RPA and FRAP). Minced pork samples (*M. semimembranosus*, 24 h *postmortem*; 1.5% salt; 10% fat) were allocated to four treatments (Control with no antioxidant; MH1 and MH2, mesquite honey from apiaries #1 and #2, at 500 ppm; and Butylhydroxytoluene at 500 ppm as a positive control (BHT-Std), cooked in a water bath (65 °C for 60 min), and assessed for pH and thiobarbituric acid reactive substances (TBARS) [5,6]. Data (n=6) were subjected to ANOVA and Tukey-Kramer's multiple comparison tests at $P<0.05$ (NCSS v11).

III. RESULTS AND DISCUSSION

As shown in Table 1, MH2 showed higher TPHC, TFC, and TTC values than MH1 ($P<0.05$). Regarding antioxidant activity, the BHT-Std showed the highest FRSA values ($P<0.05$) and no differences were detected between honey-added samples ($P>0.05$). Concerning reducing power activities, the BHT-Std showed the highest FRAP and RPA values ($P<0.05$), and the comparison between honey-added treatments indicated higher values ($P<0.05$) for MH2. As depicted in Figure 1, meat samples treated with antioxidants did not vary in pH (MH1 = MH2 = BHT-Std; $P>0.05$) whose values were slightly higher than their control counterparts ($P<0.05$). Also, MH2 showed the lowest TBARS values as compared

to other treatments (P<0.05). In agreement with the current results, it has been evidenced that honey exerts antioxidant properties associated to the presence of phenolic, flavonoid,s and tannins [4,5]. In addition, it has been reported that the inclusion of Japanese honey species in fresh beef, pork, and chicken reduced the development of oxidative compounds [7].

Table 1 – Polyphenol's content and measurements of antioxidant activity of Mesquite honey.

Item	TPHC	TFC	TTC	FRSA	FRAP	RPA
MH1	102.50±2.88^{a}	46.67±1.37^{a}	8.67±0.52^{a}	24.17±0.75^{a}	0.05±0.01^{a}	0.11±0.01^{a}
MH2	119.67±1.37^{b}	99.08±0.92^{b}	12.10±0.91^{a}	24.33±1.21^{a}	0.10±0.01^{b}	0.15±0.01^{b}
BHT-Std.	ND	ND	ND	87.33±0.52^{b}	1.04±0.05^{c}	0.82±0.02^{c}

ND: No determined.

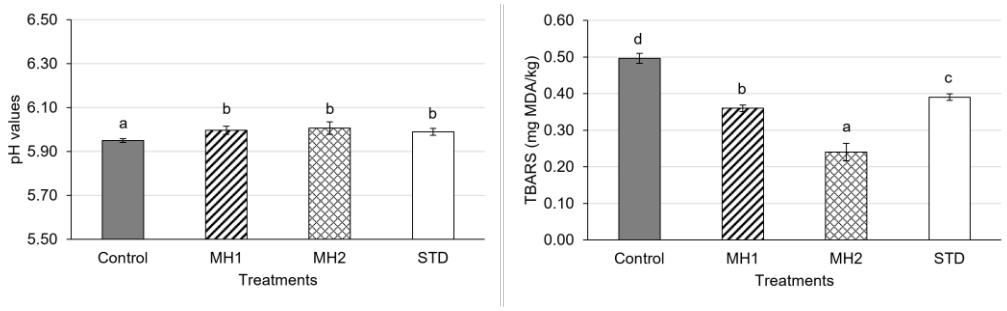

Figure 1. Effect of treatment on pH and TBARS values of cooked minced pork.

IV. CONCLUSIONS

The polyphenol content of the mesquite honey collected in Northwestern Mexico is influenced by its provenance. Mesquite honey is a source of antioxidant compounds that deserves to be proposed as a potential, natural additive for pork products but it should be noted that in this experiment its resultant antioxidant activity is far from being comparable to that of the BHT.

ACKNOWLEDGEMENTS

Rey David Vargas-Sánchez gratefully acknowledged the fellowship received from CONAHCYT "Investigadoras e Investigadores por México" Program. Authors also thank CIAD for support through "Proyecto Semilla #10735".

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Innovative Cloth Sampling Mitt to Improve Pathogen Detection for Turkey Carcasses

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I. INTRODUCTION

Sampling for detection of foodborne pathogens is a key component of food safety plans for turkey processors. We have developed a more robust and representative sampling device using a spunbond polymer cloth and validated it for various approaches to sampling beef trimmings for pathogen detection [1, 2, 3]. We further have validated an improved version of the sampling cloth by configuring it as a Mitt that fits on one hand to improve the ease of sampling beef trimmings [4]. In the current experiments the application of the sampling Mitt for use on turkey carcasses was evaluated. The objective was to determine whether Mitt sampling was at least as effective as currently used methods for monitoring food safety processes.

II. MATERIALS AND METHODS

Carcass sampling at rehang and post-chill locations in the process line was evaluated by comparing matched samples of standard cellulose sponge sampling (100^2cm x 2 locations) to a Mitt sample of a half carcass (left: 1 Mitt for *Salmonella* and right: 1 Mitt for *Campylobacter*). Mitt sampling included vigorous rubbing for 30 sec for each side of the Mitt for rehang or 30 sec on outside and 30 sec on inside for post-chill. On each of 3 days at 3 different plants, 12 rehang and 11 post-chill matched samples were collected for a total of 108 rehang and 99 post-chill observations per sampling method.

Samples were transported on cold packs overnight to the lab where 200 mL mEHEC broth (MilliporeSigma, Burlington, MA) prewarmed to 42°C was added to the mitt samples. 30 mL of rinsates were combined with 30 mL nBPW warmed to 42°C and mixed well. Samples were homogenized by stomaching for 30 sec on speed setting 7 with a BagMixer 400 (Interscience, Woburn, MA) then 2.5 mL of homogenate was removed from each sample for aerobic plate count (APC) analyses. Samples were then incubated for 12 h at 42°C and then held at 4°C until analysis. Analyses performed on enrichment broths were prevalence by PCR for the pathogen *Salmonella*: PCR for *inv*A gene found in *Salmonella*-like organisms, and PCR for pathogen index targets representative of STEC-like and *Salmonella* like organisms (Hemolysin, intimin, heme receptor [*chu*A], adhesion siderophore [*iha*A], H7, *tet*A and *tet*B genes, O group: data were obtained from three individual, non-STEC-specific, *E. coli* O serogroups: O113, O117, and O146, and generic *E. coli*. The pathogen index targets were chosen to be representative of pathogenic bacteria without being specific for any pathogen. These targets allowed for more relevant data collection as opposed to indicator counts, but did not convey any regulatory significance. For *Camplylobacter* 25 mL Hunt Broth was added to the sponge samples and 100 ml to the Mitt. Samples were enriched at 42°C for 24h under microaerophilic conditions and 3M MDA used to detect (3M, St. Paul, MN).

Enumeration data were calculated on a per-sample basis and reported as log CFU/sample. APC data were analyzed using a t test with the probability level at $P \leq 0.05$ (Prism, GraphPad Software, La Jolla, CA). Prevalence data were tallied as positive or negative for the specific PCR pathogen index targets and reported as the proportion of positive samples. Prevalence data were analyzed with a two-sided Fisher's exact test using Prism 10 (La Jolla, CA).

III. RESULTS AND DISCUSSION

At rehang and post-chill, the Mitt had higher ($P < 0.05$) recoveries of aerobic plate counts than the cellulose sponge method (Table 1). The Mitt had higher ($P \leq 0.05$) recoveries of *Campylobacter* at rehang and post-chill than the cellulose sponge method (Table 1). For *Salmonella* recovery at rehang, the Mitt had a higher ($P \leq 0.05$) prevalence than the cellulose sponge method. *Salmonella* was detected in only one sample post-chill, hence no analyses could be performed. At rehang, the prevalence of most of the pathogen index targets exceeded 80% lowering their utility in comparative analyses (20-80% provides best range for comparisons). However, the trends were similar to the results of the indicator counts and pathogen prevalence where the Mitt had higher ($P \leq 0.05$) recovery than the cellulose sponge method for tet resistance and H7 genes. The results of the O serogroup assay were within the 20% to 80% prevalence range preferred for analysis and while the Mitt had the higher numerical prevalence, it was not different statistically ($P > 0.05$) from the cellulose sponge method (Table 1). At post-chill, the Mitt had consistently higher ($p \leq 0.05$) target recoveries than the cellulose sponge (Table 1).

Table 1 – Comparison of cellulose sponge and Mitt for sampling turkey carcasses for pathogen detection.

Sample	APC, log CFU/sample	*Campylobacter* %	*Salmonella* %	*E. coli* %	Vir %	Tet %	O groups %	H7 %
Rehang								
Sponge	5.7[b]	44.9[b]	13.9[b]	94.4[a]	99.9[a]	93.8[b]	39.8[a]	76.9[b]
Mitt	6.7[a]	89.3[a]	28.7[a]	99.1[a]	100[a]	100[a]	52.8[a]	94.4[a]
Post-chill								
Sponge	3.1[b]	8.1[b]	0.0	56.6[b]	40.4[b]	46.6[b]	10.1[a]	5.1[b]
Mitt	3.7[a]	23.4[a]	1.9	96.0[a]	90.9[a]	89.8[a]	17.2[a]	45.5[a]

IV. CONCLUSION

These data demonstrate that Mitt sample collection would provide at least as good if not better performance for recovering bacteria and detecting pathogen contamination for turkey carcasses as the existing sponge method. The Mitt method samples more surface area and provides a more robust, representative sample when compared with the sponge sampling method. Furthermore, the flexibility of the Mitt provides opportunities to design sampling strategies to enhance process control monitoring by sampling multiple carcasses with one Mitt and to use the Mitt for sampling turkey parts such as wings or parts destined for ground product.

ACKNOWLEDGEMENTS

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CLOSTRIDIUM SPOROGENES PA3679 SPORES GERMINATION IN LOW-COST SHELF-STABLE BOLOGNA-TYPE PRODUCT AS AFFECTED BY REPLACEMENT OF NaCl BY KCl

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I. INTRODUCTION

Low-cost Bologna-type product stuffed in impermeable casings reaches high production volumes in Brazil. It is allowed to use up to 60% mechanically deboned chicken meat (MDCM), 10% edible offal and pork or poultry skin as raw materials. This product has been marketed at room temperature for a long time. In 2015, the Meat Technology Center of the Food Technology Institute established a Brazilian protocol for assessing the microbiological safety of Bologna marketed at room temperature in the country. The results showed that water activity was the most significant barrier in preventing the germination of spores of *Clostridium sporogenes* PA 3679, used as a surrogate for *Clostridium botulinum*, in pasteurised products containing 150ppm of nitrite and 400 ppm of sodium erythorbate. This study supported the Ministry of Agriculture, Livestock and Supply (MAPA) in establishing the maximum value of water activity (Aw) as 0.955 [1]. Partial replacement of sodium chloride by KCl is an usual strategy to decrease the sodium content of meat products [2] and the adjustment of the water activity is carried out taking into account the maintenance of the ionic strength in the system. It is unknown the impact of this replacement in preventing the germination of spores of the genus *Clostridum*, a microbiological hazard in this type of meat product. The aim of this work was to evaluate the impact caused by the replacement of sodium chloride by potassium chloride on the germination of *C. sporogenes* PA 3679 in Bologna-type product at 0.95 water activity.

II. MATERIALS AND METHODS

C. sporogenes PA3679 (ATCC 11437) was used as a surrogate for *C. botulinum*. The spore suspension was prepared according to Mah et al. [3] and contained approximately 10^5 spores/ml. In order to calculate the brine concentration corresponding to the different values of water activities, a standard batter containing MDCM (60%), pork skin (12%), pork kidney (1%), pork liver (1%), pork trimmings 70/30 (16.4%), texturized soy protein (3.5), tapioca starch (5.0%), sugar cane (0.6%), sodium tripolyphosphate (0.35), sodium acid pyrophosphate (0.15%), sodium nitrite (0.015%). The moisture of the standard batter was determined (58,97%) to calculate the amounts of KCl and NaCl to be added in order to reach the target water activities. The study comprised five treatments with different water activities (0,95, 0,96 and 0,97) adjusted with KCl (K95, K96, K97) or NaCl (N95, N97). The amounts of NaCl were calculated following Krispien et al., (1979) [4] and the corresponding amounts of KCl were calculated based on the molar concentration. Sodium erythorbate (0.04%) was added at the final comminution process in cutter. The data was submitted to one-way analysis of variance (ANOVA) and Tukey's test (V.9.1, SAS Institute, Cary, NC). Results were considered significant at $P<0.05$.

III. RESULTS AND DISCUSSION

Spore germination was observed after 15 days of storage at 35ºC in treatments N97, K95, K96 and K97. At 30 days of storage, germination was confirmed in the K95 treatment. There was no germination in the N95 treatment during the entire period, confirming the previous results. After 30 days of storage, the counts reached 6.3 log CFU/g in the K95 treatment, but remained unchanged in the N95. In the other treatments (N97, K96 and K97) the counts were not performed after 30 days of storage since at 15 days they have already showed spores germination (Table 1).

Table1. *C. sporogenes* counts (log CFU/g) in inoculated Bologna-type product during storage at 35°C.

	Storage time (days)					
	1*		15		30	
	Spores	Spores and vegetative cells	Spores	Spores and vegetative cells	Spores	Spores and Vegetative cells
N95	1.99 ± 0.07 h	2.07 ± 0.10 gh	2.57 ± 0.20 fgh	2.53 ± 0.46 fgh	2.65 ± 0.20 fgh	2.44 ± 0.17 fgh
N97	1.95 ± 0.08 h	2.16 ± 0.23 gh	7.46 ± 0.55 ab	8.18 ± 0.18 a	ND	ND
N96	2.02 ± 0.10 h	2.00 ± 0.10h	2.72 ± 0.22 fgh	5.08 ± 0.28 d	ND	ND
K95	2.13 ± 0.38 gh	2.17 ± 0.26 gh	2.94 ± 0.54 fg	4.06 ± 1.46 e	3.20 ± 0.45 ef	6.01 ± 0.86 c
K96	1.99 ± 0.12 h	2.16 ± 0.25 gh	6.06 ± 0.29 c	7.12 ± 0.20 b	ND	ND
K97	1.94 ± 0.08 h	2.19 ± 0.18 gh	7.54 ± 0.23 ab	8.27 ± 0.40 a	ND	ND

*Before incubation, 24 hours after processing; Mean and standard deviation (n=6); ND = non determined (germination occured on day 15); N95 (aw-0.95/NaCl); N97 (aw-0.97/NaCl); K95 (aw-0.95/KCl); K96 (aw- 0.96/KCl); K97(aw-0.97/KCl) Values with different superscripts are significantly different ($p < 0.05$).

IV. CONCLUSION

The water activity value of 0.95 constitutes a barrier to the germination of spores of *C. sporogenes*, provided that the salt used is sodium chloride. However, when sodium chloride is completely replaced by potassium chloride, spores' germination occurs. Although the total replacement of sodium chloride is not practiced by meat processors due to sensory issues, it is necessary that the reformulation of this type of product includes a challenge test to assess microbiological safety.

ACKNOWLEDGEMENTS

The authors thank CNPq for the doctoral scholarship of Suzana Eri Yotsuianagi.

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Enumeration of Microbial Indicators in Beef Trimmings using a MicroTally Mitt vs MicroTally Cloth Samples

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Abstract: A study was conducted to evaluate the performance of two sampling methodologies of testing for microbiological indicators in beef combos. The methodologies being compared were a singular MicroTally cloth, and a MicroTally mitt fitted to slide over the hand. Combos were swabbed either before or after treatment with an antimicrobial dip, and samples were tested for Aerobic Counts, Enterobacteriaceae Counts, and Escherichia coli Counts using the TEMPO® system. There were no statistically significant difference among counts ($p > 0.05$) achieved from either the cloth or the mitt. This observation was for both sampling done pre and post treatment, as both cloth and mitt counts were similar, regardless of when sampling occurred in the production process. The data obtained from this study regarding the quantification of indicators is applicable to beef producers internationally as a method of maintaining consistent sampling for product imported and exported globally.

Purpose: In the United States, the Food Safety Inspection Service recently changed from the use of N60 excision testing to demine if 2000 lb beef combos were contaminated to the use of a 2-minute cloth test using a MicroTally cloth. The method is nondestructive and takes less time than the excision sample. Recently, a Mitt was developed that can be placed onto the hand to sample the beef combos [1]. However, a comparison of these two methods has not been evaluated in beef combos. This in-plant study was conducted to determine the effectiveness of the two methods for microbial sampling of beef trimmings in 2000 lb combos. The samples were collected from containers containing cuts of beef in a slaughter plant in the midwestern United States. This study is important to not only US processors, but also to international markets given the fact that any product imported into the United States could be sampled using this method. Achieving a more accurate reflection of the microbiological content of beef sampled at the slaughter plant is critical to ensuring safety for both producers and consumers.

Methods: Three repetitions were performed on different days of processing. Ten swabs and ten mitts were each used for each repetition, and samples were obtained from 10 different combos (~2,000 lbs) of beef trimmings. Combos were divided in half visually, one half of the combo trim was wiped with the cloth for one minute, while simultaneously the other half was wiped with mitt for one minute. Once the minute elapsed, subjects collecting the samples switched sides, and the other half of trim was wiped with cloth and mitt for one minute again. The standard testing time for both the cloth and the mitt is two minutes according to manufacturer instructions. Samples were transported back to the laboratory and tested for Aerobic Counts, Enterobacteriaceae Counts, and Escherichia coli Counts using the TEMPO® system. Statistical analysis was performed to determine if there is a difference of data collected between cloth and mitt based on significance level of 95% for the study.

Results: Aerobic Counts, Enterobacteriaceae and *Escherichia coli* counts were not statistically different between the two methods according to the Wilcoxon Test Analysis ($p = 0.81$, $p = 0.53$ and $p = 0.36$ respectively). A linear regression was performed to account for the variation between

repetitions. The linear model for Enterobacteriaceae counts showed the best fit for the model (R^2 = 0.91) with intercept and slope of 0.090 and 0.909 respectively, followed by AC with intercept and slope of 0.4913 and 0.814 respectively and R^2 of 0.607. For *Escherichia coli* count, the intercept and slope calculated were 1.218 and 0.566 respectively. In all cases, the slopes were not statistically significant ($p < 0.05$), and only for EB the intercept was statistically significant ($p = 0.01$).

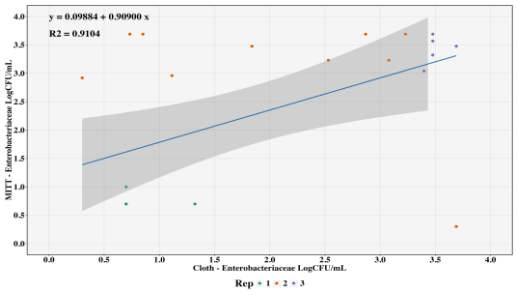

Figure 1. Linear Model for EB counts.

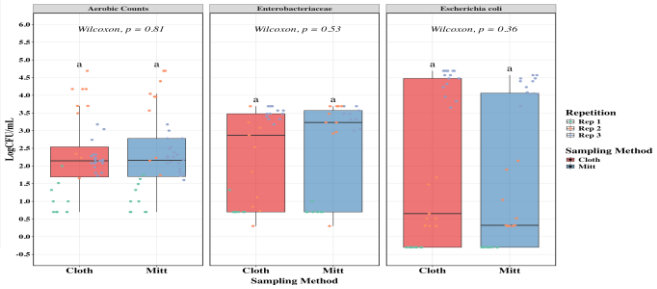

Figure 2. Wilcoxon test for all microorganisms

Conclusion: The mitt and cloth proved to have no statistical differences according to the Kruskal Test regarding the numbers of indicator bacteria detected in beef combos. The high variation within the data sets can be attributed to the fact that a repetition was done post treatment with an antimicrobial dip, while others were done before treatment. Additionally, sample collection was done in a plant enviornment where natural varation occurrs. Lower amounts of pathogens were detected from samples taken post-treatment, but the levels shown were comporable between cloth and mitt. Enterobacteriacae counts showed the best fit [2], followed by Aerobic counts, but further repetitions would be needed for *E.coli* counts to conclude methodologies will recover similar counts in all samples.

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THE EFFECT OF TEMPERATURE ON THE ABILITY TO FORM BIOFILMS BY THE FOOD PATHOGEN *L.MONOCYTOGENES*

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I. INTRODUCTION

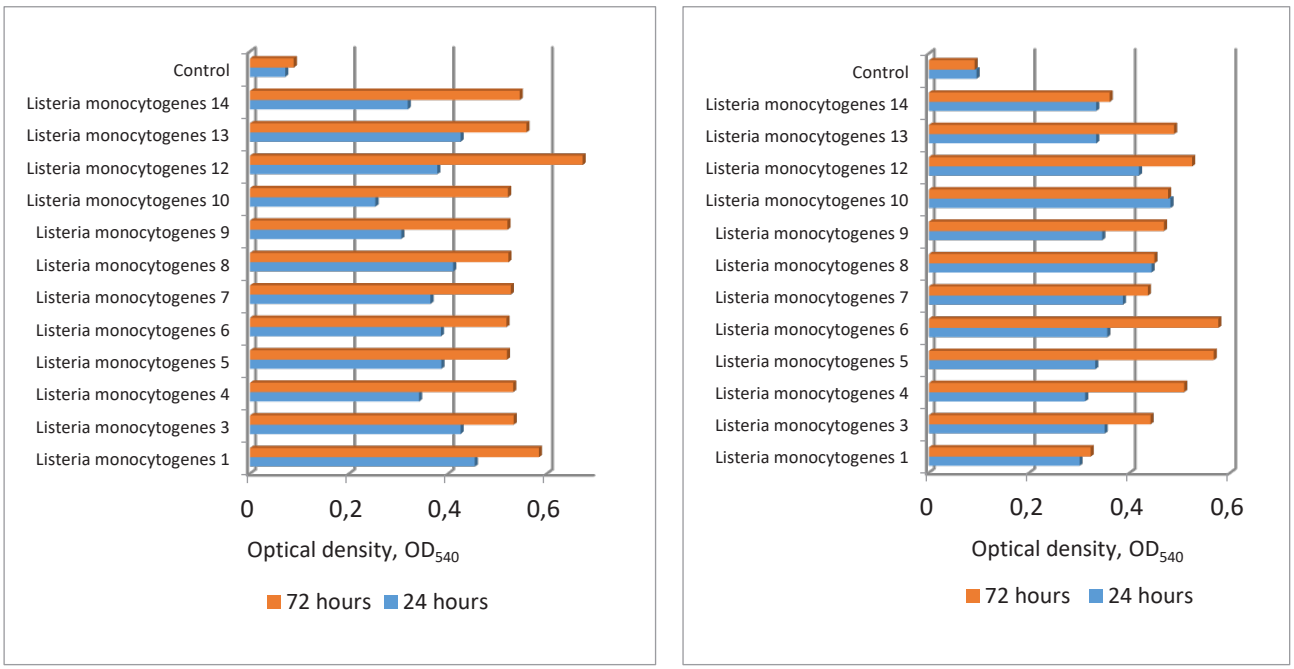
L. monocytogenes is capable of forming biofilms, which allows it to attach to the surfaces of equipment in food processing plants made of stainless steel, high-density polyethylene and glass [1]. Biofilm formation increases the resistance of *L. monocytogenes* to adverse conditions, including disinfectants used in enterprises, which makes it difficult to completely destroy [2]. Most studies focus on the formation of biofilms by pathogens at temperatures optimal for their growth, which are not typical for food enterprises. The purpose of this work was to evaluate the ability of Listeria monocytogenes to form biofilms at various temperatures, including those typical of food processing environments.

II. MATERIALS AND METHODS

Twelve strains of *Listeria monocytogenes* isolated from various objects of the production environment of meat processing and poultry processing enterprises were selected as research objects. All strains were tested for their ability to form biofilms in monoculture on polystyrene surfaces for 72 hours at 37 °C, the optimal growth temperature of the studied microorganisms, and at 4 °C, the low positive temperature characteristic of premises in food enterprises. The ability to form biofilms was studied in vitro in microtiter plates. A nighttime broth culture of bacteria was diluted 1:100 in LB broth (Becton Dickinson, USA) and 150 µl were added to the wells of a 96-well flat-bottomed polystyrene tablet (Corning, USA). After incubation, the planktonic cells were removed and the biofilms were stained with a solution of crystalline violet (Servicebio, China) with an exposure of 1 hour, followed by the addition of 96% ethanol to extract the dye bound to the biofilms. The optical density of the dye extracted with alcohol was measured on a photometer at a wavelength of 540 nm. Wells filled with sterile broth served as a control. The excess of the optical density of the crystalline violet over the control indicated the formation of biofilms by bacteria. The ability of strains to form biofilms (respectively, and strains as producers of biofilms) was classified using the following scale: no biofilm formation (OD540 = 0) → very weak (0 < OD540 < 0.2) → weak (0.2 < OD540 < 0.4) → strong (0.4 < OD540 < 1.0) → very strong (OD540 > 1.0).

III. RESULTS AND DISCUSSION

After 24 hours, *Listeria monocytogenes* formed persistent biofilms on the polystyrene surface. The ability to form biofilms varied among the strains, with temperature and time being of great importance. It is believed that a low positive temperature (+4°C) is a deterrent to the growth of microorganisms, however, the data obtained indicate that there is no negative impact on their ability to form biofilms. After 24 hours of incubation, 58% (7/12) of the ability to form biofilms at 37 ° C and 4 ° C differed by no more than 0.04 units. optical density at OD_{540} (Figure 1), which indicates the insignificance of the influence of low positive temperature as a deterrent factor for biofilm formation. At the same time, in three strains of *Listeria monocytogenes* 10, *Listeria monocytogenes* 11 and *Listeria monocytogenes* 15, the rate of biofilm formation was higher at 4 °C than at 37 °C during the first 24 hours.



a) 37 °C b) 4 °C

The intensity of formation of Listeria biofilms at different temperatures

At 37 ° C, an increase in incubation time to 72 hours contributed to the formation of a denser biofilm compared to 24 hours. At 4 °C for 72 hours, the formation of biofilms by *L. monocytogenes* microorganisms occurred less intensively, however, the positive dependence of the intensity of biofilm formation on an increase in incubation time remained in most strains (9/12). In three strains (*Listeria monocytogenes* 10, *Listeria monocytogenes* 8, *Listeria monocytogenes* 1), the duration of incubation did not affect the intensity of biofilm formation. The data obtained indicate a high intraspecific heterogeneity of strains in their ability to form biofilms under the same conditions. Although some authors reported a correlation between lineage and film forming ability [3], other results did not support these findings [4], which is also illustrated in our study.

IV. CONCLUSION

As a result of the conducted studies, the ability to form biofilms of pathogenic L. monocytogenes was shown at both 37 °C and 4 ° C. The low positive temperature (4 °C) was not a limiting factor in the ability to form biofilms. In addition, intraspecific features of the strains were noted for their ability to form biofilms under the same conditions. The results highlight the critical importance of implementing effective biofilm control strategies to ensure food safety.

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CO_2 Emitter Pads as an Alternative to Gas Flushing: Microbial and Meat Quality Evaluation

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I. INTRODUCTION

Modified atmosphere packaging (MAP) is a common food preservation tool, and it has found wide-spread application within the meat industry. The choice of packaging gas varies depending on the nature of the product, but enhanced levels of CO_2 (20 to 30%) are commonly used to provide an extended microbial shelf-life of fresh meat. One factor limiting the use of MAP in smaller locations is the capital cost of suitable gas-flush packaging equipment. A strategy that may prove more useful in these sorts of situations is the use of CO_2 emitter pads. Inclusion of an absorbent pad in packaged meat is a common practice as it absorbs the drip produced by meat during storage [1]. The CO_2 emitter pad concepts involves using this drip to cause a chemical reaction resulting in CO_2 release. Typically the reaction involves citric acid and sodium bicarbonate reacting together when exposed to water or meat drip, resulting in CO_2 production.

There are a number of parameters that could affect the suitability of CO_2 emitter pad technology in the meat industry, including the time taken to reach the target CO_2 level, maintenance of the target CO_2 level, impact on packaging performance (package swell or collapse) and the effect that the CO_2 level that is achieved has on meat quality attributes. To address these points, this study compares the effect of two types of CO_2 active pads (absorbs moisture, CO_2 production) with passive pads (absorbs moisture, no CO_2 production) used with conventional gas flush technology.

II. MATERIALS AND METHODS

The meat used in the study were from bovine semitendinosus (2 cm thick, approximately 250 g). The active (CO_2 producing) pads were: Vartdal Plast (VP; 10 cm ×15 cm) and McAirlaid's (MA; 8 cm ×13 cm). The packaging treatments were: P1, 1x VP pad with air; P2, 1x MA pad with air; P3, 2x MA pads with air; P4, 1x passive pad with air; P5, 1x passive pad with 30% CO_2, 20% O_2, 50% N_2; P6, 1x passive pad with 30% CO_2, 70% O_2. The appropriate pad were placed in the bottom of clear polypropylene trays (170 x 223 x 40 mm; Cryovac® Barrier Trays). Beef slices were weighed and placed on the pad(s). The trays were transferred to the gas packer (T-200 Multivac), flushed with the appropriate gas mixture, and sealed with biaxially oriented polyamide/ethylene vinyl alcohol copolymer polyethylene film (LID-AEE-AP-45, Multivac). The performance of the different packaging system were assessed using headspace gas measurement (O_2 and CO_2; Quantek Model Q2, USA), meat pH (surface meat sample homogenized in distilled water and measured [Oakton pH 700 Benchtop Meter]), meat color (CIE *Lab* system; Chroma Meter, CR-400, Konica Minolta, Japan), meat drip loss (weight change), and total aerobic microbial counts (enumerated using 3M™ Petrifilm Rapid Plates codes 6478). Samples were analyzed on days 0, 3, 7, 10, 14, 17 and 21. Triplicate samples were analyzed for each combination of packaging treatment and sampling time. The experimental design consisted of 108 trays (comprising 6 treatments × 6 sampling times × 3 replicates).

III. RESULTS AND DISCUSSION

The headspace gas composition changed during the storage time (Figure 1), reaching a maximum of approximately 60% CO_2 in emitter pads P1 and P3, and 50% in emitter pad P2. Importantly, the CO_2 concentration of P1 and P3 had exceeded 30% before the earliest sampling time (day 3), indicating that the emitter pad technology was capable of rapidly producing a microbially inhibitory environment. Further work on the production of CO_2 in the first few days of storage would clarify the time taken for inhibitory levels of CO_2 to be achieved.

The effect of meat contact with the pad or the headspace gas was considered for pH and color. As expected, the meat pH changed during storage, showing an initial increase, and then declining. The pH of the passive pad samples showed minimal difference in pH between the two surfaces. In contrast, the emitter pads showed larger differences, with the pad contact pH being higher than the gas contact surface.

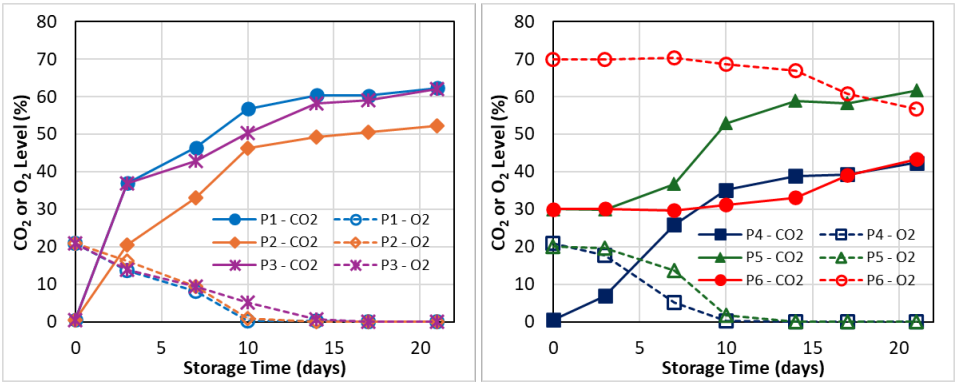

Figure 1. Levels of CO_2 and O_2 during chilled storage (4 °C) for up to 21 days in packages containing either active or passive pads.

Meat color showed a similar pattern (data not shown), with passive pads showing no significant difference between gas and pad contact surfaces for a* and b*. However, active pads showed significant differences with the active pad contact surface having a higher a* and lower b* than the gas contact surface.

The aerobic plate counts (APC) of all samples started at the same level (2.33 ± 0.15 Log cfu/cm^2). The P1, P3 and P5 controlled APC numbers with similar effectiveness (Table 2). P4 (air headspace) and P6 (30% CO_2, 70% O_2) saw less effective control of APC numbers, and had the highest counts at the end of storage. Interestingly P2 proved less effective initially (days 1 and 7), which may reflect the slower increase in CO_2 level in this treatment.

Table 1. The pH of meat surfaces in contact with the gas or pad. Selected days presented for brevity.

		Time (day)			
		0	7	14	21
P1	Gas	5.3 ± 0.02	5.7 ± 0.03	5.8 ± 0.03	5.7 ± 0.02
	Pad	5.3 ± 0.02	5.9 ± 0.02	6.0 ± 0.05	5.7 ± 0.03
P2	Gas	5.3 ± 0.02	5.7 ± 0.02	5.8 ± 0.03	5.7 ± 0.01
	Pad	5.3 ± 0.02	5.7 ± 0.03	5.9 ± 0.01	5.6 ± 0.03
P3	Gas	5.3 ± 0.02	5.7 ± 0.05	5.8 ± 0.02	5.7 ± 0.04
	Pad	5.3 ± 0.02	5.7 ± 0.02	5.9 ± 0.03	5.7 ± 0.04
P4	Gas	5.3 ± 0.02	5.7 ± 0.06	5.6 ± 0.05	5.5 ± 0.01
	Pad	5.3 ± 0.02	5.7 ± 0.05	5.6 ± 0.04	5.5 ± 0.02
P5	Gas	5.3 ± 0.02	5.6 ± 0.03	5.7 ± 0.02	5.5 ± 0.03
	Pad	5.3 ± 0.02	5.6 ± 0.03	5.7 ± 0.06	5.4 ± 0.03
P6	Gas	5.3 ± 0.02	5.6 ± 0.01	5.3 ± 0.03	5.1 ± 0.02
	Pad	5.3 ± 0.02	5.6 ± 0.01	5.3 ± 0.01	5.1 ± 0.02

Table 2. Aerobic plate count (Log cfu/cm^2) for samples during storage. Selected days presented for brevity.

	Time (day)			
	3	7	14	21
P1	2.73 ± 0.08	2.82 ± 0.19	5.05 ± 0.19	5.52 ± 0.12
P2	3.03 ± 0.18	3.12 ± 0.45	5.16 ± 0.10	5.21 ± 0.45
P3	2.44 ± 0.30	2.81 ± 0.27	5.11 ± 0.12	5.71 ± 0.43
P4	4.25 ± 0.41	4.32 ± 0.16	5.77 ± 0.11	6.28 ± 0.12
P5	2.52 ± 0.11	2.62 ± 0.11	4.71 ± 0.12	5.74 ± 0.31
P6	3.50 ± 0.14	3.48 ± 0.24	4.75 ± 0.41	6.44 ± 0.06

IV. CONCLUSION

The emitter pads used rapidly increased the CO2 levels in the samples, with P1 and P3 reaching over 30% CO_2 by day 3 of storage. P1, P3 and P5 had comparable APC numbers throughout the study. However, other attributes (pH, color) responded differently in the emitter pad samples compared to the passive pad samples. In particular, the meat surface in contact with the emitter pad differed from the surface in contact with the headspace gas. This issue did not occur with the passive pads. To fully develop this technology for application to beef, further refinement of the pad technology along with changes such as in the film composition to better manage the CO_2 levels achieved.

ACKNOWLEDGEMENTS

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ISOLATION OF BACTERIOPHAGES FOR BIOCONTROL OF PATHOGENIC MEAT BACTERIA

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I. INTRODUCTION

Contamination of meat products can occur mainly due to the presence of microorganisms belonging to the natural microbiota of animals intended for consumption. These microorganisms can compromise the microbiological quality and safety of meat products by system failures or abuses during food animal production, product processing and distribution, and preparation for consumption, as well as by consumption habits [1]. It is essential to promote research and development of techniques to guarantee the safety of meat products, in relation to the presence of chemical residues harmful to the human body and pathogenic microorganisms, including, the control of resistant bacteria to chemical agents that can cause damage throughout the production chain. In this scenario, a promising possibility arises through the use of bacteriophages or phages, which are viruses that can act in the biocontrol of pathogenic bacteria. Phages have interesting properties for the food industry because they specifically infect the bacterial host, replicate in the presence of this host, and are widely distributed in the environment, as well as in many types of food [2]. Phages can be used prophylactically, therapeutically and to sanitize surfaces and for spraying on meat products or packaging. Unlike antibiotics, they offer greater versatility in choosing phage cocktails and treatments to be used in the meat production chain. Thus, due to the great advantages of using biocontrol through phages, its application in meat products is a promising and valuable tool to help control undesirable microorganisms present in products of animal origin.
This work aims to isolate bacteriophages with inhibitory action on relevant pathogenic bacteria which can be found in meat and meat products.

II. MATERIALS AND METHODS

The bacterial strains used in this study belong to the CTC – ITAL laboratory collection and consisted of type strains and environmental sources isolates of *Listeria monocytogenes, Salmonella typhimurium*, *Escherichia coli* O157:H7, and *Staphylococcus aureus*. Swab samples from contact surfaces were collected from the pipelines of the sewer and drains of the meat processing plant of CTC-ITAL, following standard methods of identification. Bacterial strains were routinely grown in TSB culture medium incubated at 37°C, and permanent stocks were stored at -80°C in 20% glycerol.
Phages were isolated through concentration with polyethylene glycol, according to [3]. For this, samples (500ml) of the environment in which animals are raised (soil from the cattle corral and lagoon containing pig waste) from the city of Pirassununga, SP, and from the sewage of a local meat processing plant from CTC/Ital, were analyzed. The samples received SM buffer and NaCl to the final concentration of 1M and were centrifuged (7,500rpm) to remove impurities. The supernatant received Polyethylene Glycol 8000 to a final concentration of 10% (w/v) and was kept at 4°C for a overnight decantation. The material was subjected to centrifugation at 7,500rpm for 20min, the pellet was resuspended in SM buffer and extracted with an equal volume of chloroform. The tubes were centrifuged at 7,500rpm for 10min and the supernatant containing the phage extract was stored at 4°C protected from light. Phages were isolated through the agar overlay method [4]. Single lysis plaques were collected with sterile picks, and added to an exponential growing bacterial culture following incubation overnight at the suitable temperature.
For the induction of bacteriophage infection, 200µL of active bacterial cultures were transferred to sterile 2mL microtubes. 300µL of phage extract was added to these tubes followed by incubation at 30°C for approximately 1h. After this period, tubes containing 5mL of semi-solid culture medium (0.7% agar) were melt, which were kept in a water bath at 44°C. Then, the bacterial suspension and phage extract were transferred to semi-solid culture medium tubes, gently homogenized and evenly distributed over the

surface of Petri dishes containing the appropriate culture media. After the media solidified, the plates were incubated at the optimum growth temperature for each microorganism for 24h and the formation of lysis plaques was evaluated.

III. RESULTS AND DISCUSSION

From the swabs collected at the meat processing plant, six strains of *L. monocytogenes* were isolated, which were included as indicators in the tests along with the type cultures. The inhibitory action of the phages could be evidenced by the formation of clear areas on the plates inoculated with *E. coli* O157:H7 ATCC 43895 or *L. monocytogenes* ATCC 7644 (Figure 1). Regarding *Staph. aureus* and *S. typhimurium*, no phages with inhibitory action on these bacteria were isolated from the samples tested. The isolated viral extracts were coded as phages A4, A7, A14, A15, and A17 and were detected in samples collected from animal breeding environment and lagoon water. *L. monocytogenes* was infected by three phages (A4, A7, and A14), while *E. coli* O157:H7 was infected by all the phages isolated. Therefore, phages A4, A7 and A14 presented an unusual trait in relation to the other phages, showing inhibitory action on both bacterial cultures. On the other hand, phages A15 and A17 showed specificity only for *E. coli* O157:H7. According to Nikolich & Filippov [5], phages infect their bacterial hosts in a specific way, and it is more common for phages to infect only one species of bacterium or a subgroup within the same bacterial species. Thus, it is important to investigate the reason for the cross-reactivity between two different genera of bacteria and these divergent phages, clarifying whether this is a real property or just a consequence of testing a phage mixture that has not yet been purified.

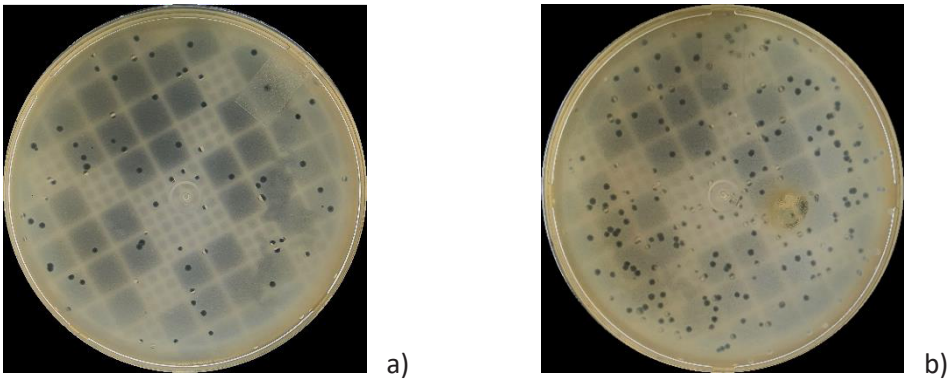

Figure 1. Agar plates with phage A7 with a) *E. coli* O157:H7; b) *L. monocytogenes*.

IV. CONCLUSION

Phages with inhibitory potential against *L. monocytogenes* and *E. coli* O157:H7 were detected, but it was not possible to obtain phages with action against *Staph. aureus* and *S. typhimurium*. It was demonstrated that isolated phages can be efficient in controlling the growth of bacteria which are harmful to the meat industry.

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"Microbiome of Healthy Beef Cattle Lymph Nodes and Digesta using 16S rRNA Gene Sequencing Analysis"

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I. INTRODUCTION

Bacterial cross-contamination during slaughter is intricate and difficult to identify foodborne pathogens due to their hidden, unseen, and intangible nature [1]. The digestive system of beef cattle and its associated organs are regarded as a primary source of microbial contamination of the final product [2]. While the digestive system is recognized as a primary source of microbial contamination [3], especially in beef processing, fecal contamination of hides is recognized as the major source of contamination by enteric pathogens such as *E. coli* O157:H7 and *Salmonella* [4]. Mann et al. [1] noted that the risk associated with lymphatic organs is low as long as tissue integrity is maintained. However, the possibility of lymph nodes remaining intact during carcass processing and ground beef production is not feasible. Despite efforts, Webb et al., reported a full characterization and description of the burden across multiple variables on the prevalence of *Salmonella* in the pre-scapular lymph nodes (PLN) from feedlot-fattened (FF) cattle from a region during the warmer season, the data reveal a significant prevalence rate of 31.1% [5]. Despite recent progress in high-throughput sequencing, the bacterial microbiome composition in various animal niches has remained unexplored in beef cattle lymph nodes. We hypothesize that bacterial communities can be described in the lymph nodes of healthy slaughtered beef cattle. This study aims to estimate the bacterial biodiversity of each PLN and MLN, comparing them with potential bacterial translocation from the digesta (fecal and cecal content) from beef cattle at harvest. This would constitute the first study to use DNA and RNA-based methods to investigate the diversity of the bacterial community in peripheral and mesenteric lymph nodes of beef cattle, offering insights pointing to pathogenic bacterial strains detected in the LNs derived from the Gastrointestinal Tract (GIT) and have therefore impact on risk assessments in slaughterhouses regarding a possible distribution of spoilage, foodborne and zoonotic pathogens.

II. MATERIALS AND METHODS

Beef lymph nodes (LNs) tissues were sampled at a commercial harvest facility in the Midwestern region of the USA. A total of 38 lymph nodes (LNs) were sampled: inferior-ileocecal (IILNs, n= 8), popliteal (PLNs, n= 8), subiliac (SLNs, n= 8), superior-ileocecal (SILNs, n= 8), and mandibular (MLNs, n= 6). After removal LNs were purified from loose tissue and fat residues, and the surface was disinfected by rigorously dipping into 70% ethanol. The digesta samples were constituted of 8 cecal content and 8 fecal samples. All beef cattle included in this study were market animals originating from commercial feedyards in the USA. DNA from LN tissues was extracted using Qiagen DNeasy blood and tissue kit. We utilized total genomic DNA extracted from bacterial samples as the template for polymerase chain reaction (PCR) amplification. Specifically, targeting the hypervariable regions within the V3-V4 region of the 16S ribosomal RNA (rRNA) gene, a widely used marker for bacterial identification and classification. Microbial abundance and taxonomic classification were evaluated using the DADA2 and QIIME2 Package in R version 4.3.2.

III. RESULTS AND DISCUSSION

We found a total of 14,115 amplicon variant sequences (ASVs) in fecal, cecal content and LNs samples, indicating a substantial diversity of microbial species present. Firmicutes, Bacteroidetes, and Proteobacteria were the most abundant phyla in all experimental groups, which has been previously described in beef fecal samples [6]. The taxonomic classification revealed the most abundant genus

according to the relative abundance composition of bacterial communities of the fecal samples belonged to *Rombutsia* 9.06%, *Bacteroides* 8.73%, and *Oscillospiraceae* 7.66%. This was followed by cecal content samples where the most abundant genus was represented by *Bacteroides* 11.09%, *Oscillospiraceae* 9.44%, and *Alloprevotella* 8.01%. The comparison with the most abundant genus in PLNs was represented by *Staphylococcus* 15.24%, *Enterococcus* 14.17%, and *Escherichia/Shigella* 6.87%. In contrast, MLNs most abundant genus were belonged to *Enterococcus* 45.75%, *Escherichia/Shigella* 26.73%, and *Bacillus* 4.79%. The alpha diversity comparison between groups LNs-BF and LNs-CC was according to the observed richness (p<1.075e-09), Chao1 Richness (p<1.091e-09), and Shannon diversity (p<8.177e-08). These results suggest variations in microbial diversity between different lymph node locations and digesta [7].

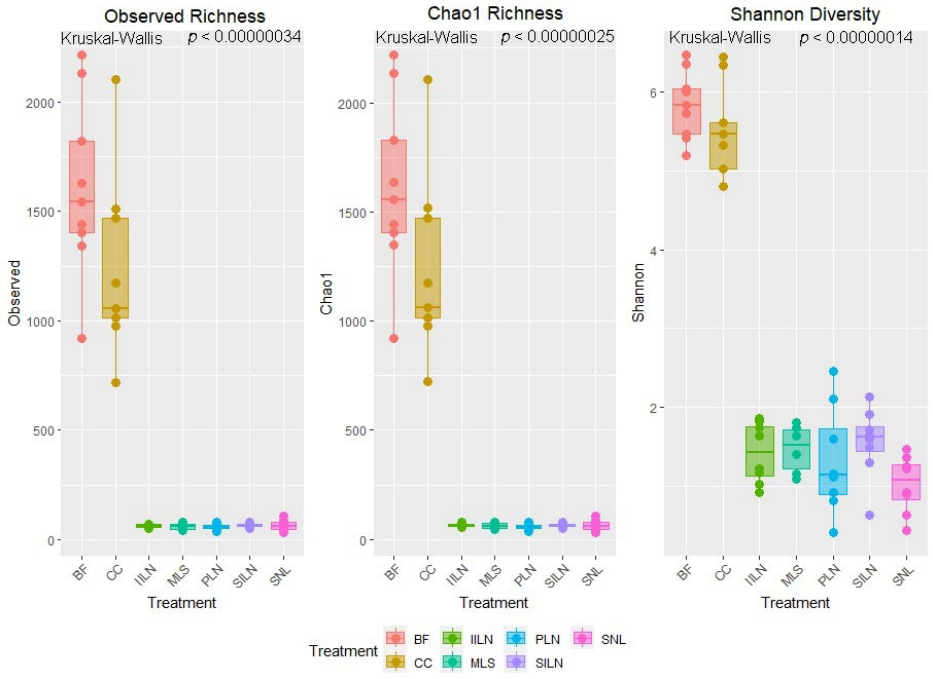

FIG 1. Alpha diversity indices of microbiome samples. Fecal and Cecal content were compared to LN groups significant differences were noted within the alpha diversity indexes (*P<0.05*).

IV. CONCLUSION

This pioneering study utilizes DNA-based methods to explore bacterial diversity in US beef cattle's peripheral and mesenteric lymph nodes. The presence of *Escherichia/Shigella* and *Clostridium* sensu stricto 1 genus and the powerful correlation of the bacterial relative abundance in the peripheral lymph nodes and digesta suggest a possible bacterial translocation and presence of potentially pathogenic bacteria from the Gastrointestinal Tract (GIT), highlighting the importance of risk assessments in slaughterhouses to prevent the spread of spoilage bacteria, foodborne, and zoonotic pathogens.

ACKNOWLEDGEMENTS

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Impact of Frozen Storage Temperatures on Ground Beef Microbial Quality

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I. INTRODUCTION

In response to escalating energy costs and growing pressure from society to be more energy-efficient and climate-smart, along with the ambitious goal set out by the United Nations Net Zero Coalition to achieving net zero emissions by 2050 [1], this study was conducted to examine how different frozen storage temperatures affect the microbial shelf life of ground beef. By improving our precision of temperature controls during frozen storage, we can address the sustainability goals of society while also preserving meat safety and quality. In 2022, the Transparency Market Research valued the global frozen meat market at 23.2 billion USD [2], thus highlighting the importance to further understand freezing impacts. The objective of this study was to evaluate the impact of frozen storage temperatures on the microbiological quality utilizing ground beef during 30 days of storage as a model to represent frozen beef in commerce in highly controlled frozen storage environments.

II. MATERIALS AND METHODS

Beef strip loins (IMPS #180; vacuum-packaged product held at 2°C for 12 days post-fabrication) were divided dorsally into smaller portions and were submerged in boiling water for 2 min, to reduce the natural microbial contamination level on the surface of the loins. The heat-exposed surface was then trimmed off and lean was separated from the fat tissue. The separated lean and fat were cubed to simulate beef trimmings. Batches of lean (90%) and fat (10%) tissue were inoculated with a mixture of six common meat spoilage bacteria [3] previously isolated from spoiled beef steaks. The 6-isolate mixture was comprised of three *Pseudomonas* spp. (*P. fragi*, *P. fluorescens*, *P. lundensis*) and three lactic acid bacteria (*Carnobacterium divergens*, *Leuconostoc gelidum*, *Lactobacillus sakei*). The inoculated trimmings (ca. 4 log CFU/g) were coarse ground twice through a 12.7 mm grinder plate. Ground beef was portioned into 100 g samples and vacuum packaged. The samples were then randomly assigned to and stored in temperature test chambers (Tenney T2C-A-F4T Temperature Test Chambers, Thermal Product Solutions, New Columbia, PA, USA) at experimental temperatures of -20.6°C, -15.0°C, and -9.4°C (-5°F, 5°F, and 15°F, respectively). On days 1, 15, and 30 of storage, samples were removed from frozen storage, thawed (4°C, 24 h), and analyzed for aerobic plate counts (APC; tryptic soy agar; 25°C, 72 h). Non-frozen samples were also analyzed for APC on day 0 to determine the ground beef inoculation level. Two trials were performed per storage temperature with five replicates per trial (n = 10). Due to the availability of only two temperature test chambers (A and B; Table 1), three experimental setups were performed, and treatments (i.e., storage temperatures) were randomized between the two units as shown in Table 1. Statistical analysis was performed using R version 4.1.2 and included block (replication) and storage temperature. Significance level was set at $\alpha = 0.05$.

Table 1 – Experimental setup with two temperature test chambers and randomization of treatments (i.e., storage temperature) for ground beef.

Temperature test chamber	Experimental setup		
	1	2	3
A	-9.4°C	-20.6°C	-15.0°C
B	-20.6°C	-15.0°C	-9.4°C

III. RESULTS AND DISCUSSION

Our preliminary findings suggest that, under the highly controlled (i.e., minimal variation of ±0.01°C) frozen conditions of our study, storage of ground beef at -20.6°C, -15.0°C, or -9.4°C did not ($P \geq 0.05$) have an impact on microbial quality (Table 2). It remains to be seen if temperatures in commercial facilities can be controlled to likewise maintain microbial quality of beef, while simultaneously increasing storage temperatures and reducing environmental impact.

Table 2 – Mean ($n = 10$) aerobic plate counts (log CFU/g ± standard deviation) of inoculated (4.35±0.07 log CFU/g) ground beef stored at -9.4°C, -15.0°C, or -20.6°C for up to 30 days.

Storage day	Storage temperature[1]		
	-9.4°C	-15.0°C	-20.6°C
1	4.34±0.05	4.32±0.09	4.33±0.05
15	4.33±0.05	4.27±0.08	4.28±0.07
30	4.30±0.03	4.28±0.05	4.28±0.06

[1]The interaction between storage temperature and storage day was not significant ($P \geq 0.05$); storage temperature and storage day main effects were also not significant ($P \geq 0.05$)

IV. CONCLUSION

No effects of storage temperature were observed on the microbiological quality of ground beef stored for 30 days. This pilot study offers valuable insights into the influence of storage temperature on microbial survival and the quality of beef products and provides a basis for adjusting, using modern cold storage technologies, frozen storage temperatures to reduce scope-3 environmental impacts of meat distribution. As cold storage facilities and meat packers strive to achieve sustainability goals, such as reaching net-zero emissions by 2050, adjusting frozen storage temperatures could potentially reduce energy consumption within facilities without compromising food safety or quality, provided that temperatures are maintained at very consistent levels. Further research is needed to validate these findings on other products.

ACKNOWLEDGEMENTS

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CONTAMINATION FROM CONDENSATION DROPLETS IN SLAUGHTERHOUSES ENVIRONMENT

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I. INTRODUCTION

Despite the importance given by audit and inspection systems to condensation in food processing industries, there are no microbiological criteria established. Previous scientific work on this subject is also rare. Considering the lack of specific legislation, and the widely accepted hazards associated to condensation drops as sources of meat contamination, the European Commission Decision 2073/2005/EC was taken as a comparative standard, which establishes Microbiological criteria for foodstuffs (expressed in CFU/cm^2) for carcasses of cattle, pigs, sheep, goats, and horses (1). This project was developed with the objective of verifying the bacteriological profile of condensation in cold rooms and cattle slaughterhouse-refrigeration facilities.

II. MATERIALS AND METHODS

The contamination profile, represented by the bacteria present in the condensation drops located at different points of the cattle slaughterhouse, was verified over a period of three weeks by carrying out samples on nine different days. Samples were collected in places with condensation, gently touching the drop with a swab, without it meeting the surface. At least two swabs were obtained from each sampled point, which were introduced into a test tube containing 5 mL of 0.1% peptone water. Drops from the same location constituted a “pool” that represented a single sample. Thus, in total, 35 samples in the form of a “pool” and 750 drops of condensation were collected.
Bacterial species and groups of indicators present in the condensation droplets in the chilling chambers (at the entrance, center and at the exit), in the quarters, and deboning room were investigated. The squeegees used to wipe away the condensation droplets were also sampled. Counts of mesophilic and psychrotrophic microorganisms and presence of *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Clostridium estertheticum* and *C. gasigenes* were carried out.

III. RESULTS AND DISCUSSION

The mesophilic count revealed contamination in 24/35 samples (69%) with variable figures. The average contamination was 0.65 log CFU per condensation drop. When only positive samples were analyzed, the average was 1.86 log CFU per drop, with only one sample - obtained from the ceiling of the cutting room - with values above 3.5 log CFU, (3.64 log). The psychrotrophic count revealed contamination in only 4/35 samples (11.43%). The average for positive samples was 2.9 log CFU/mL. Within the 750 drops of condensation analyzed, neither *Listeria monocytogenes* nor

Salmonella spp. were detected. *Escherichia coli* was identified in only two samples (5.71%), one of them from drops located on the counterweight of the saw in the cutting room and the other at the exit of the maturation chambers, on the plastic curtains located around the rail's window. In both locations, flaws were found in the hygiene and sanitation processes when samples were collected. *Clostridium estertheticum* was detected in 4/35 samples (11,43%).
Inspection and audit services consider condensation a serious hazard to food safety as drops, when falling on surfaces or food products, will carry pathogenic or spoilage bacteria, thus contaminating products. However, the results obtained in this study showed low contamination in the condensation droplets, both for pathogenic and spoilage microorganisms. The absence of *Listeria monocytogenes*, a microorganism frequently implicated as a possible contaminant of condensation droplets, can be considered as one of the most relevant results obtained in this research. *Salmonella* spp. was also not found in any sample.
Our results, indicating less contamination than previously reported by other authors (2), may be related to the efficacy of self-control programs adopted by food export industries.

IV. CONCLUSION

Results regarding contamination present in condensation droplets showed the absence of pathogens, such as *Listeria monocytogenes* and *Salmonella*, as well as low counts of mesophiles and psychrotrophs in the studied areas. Therefore, the current practice of zero tolerance for condensation, applied by health authorities, does not have a scientifically proven justification. This policy must be reviewed and complemented by improvements in cleaning and sanitation procedures, accompanied by surfaces assessment where condensation forms, aiming to ensure that they offer low risk. Our results suggested that cleaning and sanitizing methods regularly applied in areas where condensation forms kept contamination under control. However, it is essential to review these programs, increasing care and attention, aiming specifically to surfaces where condensation is frequent. The absence of these two pathogens associated with the results of low counts of indicator microorganisms suggests that the relevance granted by inspection and audit services to condensation as a hazard must be reviewed, nevertheless, control, monitoring and prevention measures must be maintained.

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Impact of Peracetic acid concentrations on microbial and quality traits of broiler meat

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I. INTRODUCTION

Poultry meat, known for its nutritional benefits and is essential for addressing global protein deficiencies [1]. However, safety concerns arise due to contamination by pathogens, often linked to the supply chain [2]. Traditional decontamination methods, such as chlorine, can negatively affect meat quality [3]. The use of peracetic acid (PAA), a safer alternative recognized for its efficacy in reducing microbial loads without compromising meat's quality attributes like pH, color, and water-holding capacity [4]. The study aims to enhance poultry meat safety and hygiene, potentially lowering chances of foodborne illness and advancing industry processing practices.

II. MATERIAL AND METHODS

Poultry breast fillets (340g ± 10g) were procured from a local wet market. The experimental design included a control, treatments with tap water and 0.015% (T1), 0.020% (T2), and 0.025% (T3) peracetic acid (PAA), applied for 30 seconds on days 0, 3, and 5, involving 135 samples in total. Fillets were treated with PAA at concentrations of 150 ppm, 200 ppm, and 250 ppm, respectively, and stored for subsequent evaluations. Microbiological assessments (Total Viable, Salmonella, Pseudomonas, and E. coli counts) followed ISO standards. Measurements included color, pH, water-holding capacity, cooking yield, and loss were recorded accordingly. Sensory attributes were rated using a 9-point Hedonic scale. Statistical analysis utilized SPSS to identify significant differences at (p<0.05) using Tukey's test.

III. RESULTS AND DISCUSSION

TVBN was highest in T3 and lowest in control and tap water treatments (p<0.01). T2 exhibited the highest cooking loss, whereas T3 had the highest cooking yield (p<0.01). Microbial analysis indicated higher Pseudomonas, E. coli, and Salmonella counts in the control and tap water treatments compared to T3 (p<0.05 for Pseudomonas; p<0.01 for E. coli and Salmonella). Total viable count (TVC) remained similar across treatments (p>0.05) but increased significantly by day 5 (p<0.01). Sensory evaluations rated T1 highest for taste, juiciness, odor, tenderness, and overall acceptability (p<0.01). Meat color analysis revealed the lowest L* values in control, higher a* in control, and higher b* in T3, T1, and tap water (p<0.01). Meat pH was notably higher in the tap water treatment (p<0.01).

Table 1: Mean *p*-values for TVBN, Cooking loss, cooking yield, WHC, pH, and microbiology among the treatments and days.

Treatment	Day	TVBN	Cooking Loss (%)	Cooking Yield	WHC	pH	TVC	Pseudomonas	E. coli	Salmonella
Control	0d	0.3±0.1^{d}	28.3±1.4^{b}	71.7±1.6^{b}	25.3±4^{bc}	6.4±0.1^{ab}	64.3±12.9	8.8±2.6^{a}	7.7±1.9^{b}	1.9±0.5^{a}
	3d	0.4±0.1^{d}	20.2±1.4^{b}	81.8±1.6^{a}	26.8±4^{bc}	6.3±0.1^{ab}	37.3±6.1	3.7±4.1	4±2.3	1±0.4
	5d	0.5±0.1^{d}	24.2±1.4^{b}	75.8±1.6^{b}	28.8±4^{bc}	6.1±0.1^{b}	93.9±4.4^{a}	11±4.1	5±2.3	1±0.4
Tap water	0d	0.4±0.1^{d}	26±1.4^{b}	74±1.6^{b}	29±4^{a}	6.5±0.1^{a}	63.6±5.7	10.9±4.2^{a}	21.4±3.6^{a}	2.4±0.6^{a}
	3d	0.5±0.1^{d}	21.8±1.4^{b}	78.2±1.6^{a}	34.4±4^{a}	6.5±0.1^{a}	37.1±3.9	3.7±4.1	12±2.3	1.7±0.4
	5d	0.7±0.1^{d}	25.5±1.4^{b}	74.4±1.6^{b}	37.4±4^{a}	6.1±0.1^{b}	67.9±5.2	16.3±4.1	19.7±2.3	1±0.4
T1	0d	0.6±0.1^{c}	29.1±1.4^{b}	70.9±1.6^{b}	23.4±4^{bc}	6.2±0.1^{b}	65.4±11.8	4.4±1^{ab}	5.6±1.6^{b}	0.4±0.2^{b}
	3d	0.6±0.1^{c}	21±1.4^{b}	79±1.6^{a}	25.3±4^{bc}	6.1±0.1^{b}	46±9.5	2.3±4.1	2±2.3	0.3±0.4
	5d	0.8±0.1^{b}	20.1±1.4^{b}	79.9±1.6^{a}	27.3±4^{bc}	6.1±0.1^{b}	43.8±10.5	5.3±4.1	4±2.3	0±0^{b}
T2	0d	0.5±0.1^{d}	29.4±1.4^{a}	70.6±1.6^{b}	21.5±4^{c}	6.3±0.1^{ab}	56.6±10.8	4±1.3^{ab}	3.8±0.9^{b}	0±0^{b}
	3d	0.8±0.1^{b}	28.7±1.4^{a}	71.3±1.6b	25.3±4^{bc}	6.1±0.1^{b}	30.4±7.1	7.7±4.1	3±2.3	1.7±0.4
	5d	0.9±0.1^{b}	27.9±1.4^{a}	72.1±1.6^{b}	27±4^{bc}	6.1±0.1^{b}	67.9±5.2	7.7±4.1	1.7±2.3	0±0^{b}
T3	0d	0.8±0.1^{b}	14.9±1.4^{c}	85.1±1.6^{a}	30±4^{a}	6.2±0.1^{b}	51.9±10.8	0±0^{b}	2.3±0.7^{b}	0±0^{b}
	3d	0.9±0.1^{b}	17.9±1.4^{c}	82.1±1.6^{a}	31.4±4^{ab}	6±0.1^{c}	30.4±7.1	1.7±4.1	0.7±2.3	0±0^{b}
	5d	1.1±0.1^{a}	25.5±1.4^{a}	75.5±1.6^{b}	35.2±4^{a}	6.1±0.1^{b}	89.7±9.8	0±0	0.7±2.3	0±0^{b}

TVBN: Total volatile basic nitrogen
WHC: Water holding capacity.

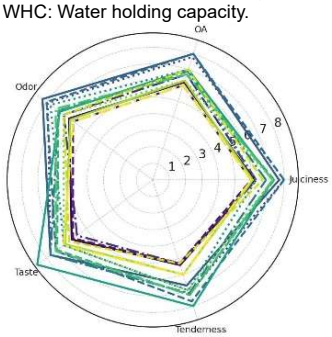

Figure 1: Radar Graph for Sensory

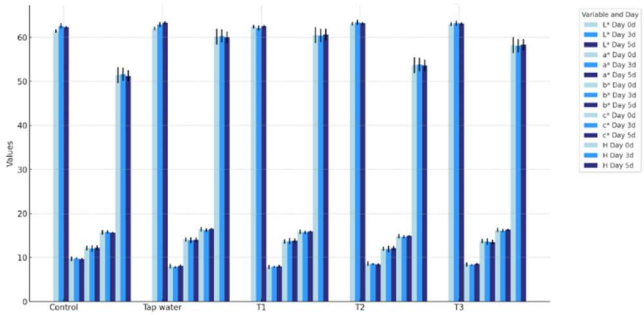

Figure 2: *L**, a* and b* values among treatment and days

IV. CONCLUSION

The study found that peracetic acid (PAA) at 200 ppm effectively enhances the physicochemical and microbiological characteristics of chicken breast fillets without altering their sensory qualities.

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HANDHELD SCANNER FOR RAPID ASSESSMENT OF HYGIENE STATUS IN MEAT SLAUGHTERING AND MEAT HANDLING FACILITIES

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I. INTRODUCTION

In meat processing facilities, the complexity of operations leads to hygiene challenges, as the entire process involves multiple stages like slaughtering, cutting, deboning, and packaging. Each stage carries distinct hygiene risks, complicating the monitoring and maintenance of cleanliness throughout the process. Additionally, the risk of cross-contamination is high due to the handling of raw meat and the use of shared equipment and surfaces, requiring meticulous control and cleaning procedures to manage. Pathogens such as *Salmonella, Listeria, and E. coli* are invisible to the naked eye and traditional microbial testing methods are often slow, delaying necessary corrective actions. Maintaining consistent adherence to hygiene standards and regulatory compliance also proves difficult across different shifts and among all workers, necessitating thorough training and monitoring to ensure protocol compliance. The current practice involves visual inspection and limited swab testing which cannot cover the size and variety of surfaces at risk. Moreover, organic residues from meat processing can form biofilms on equipment and surfaces, which are not only tough to remove but also harbor pathogens, requiring effective cleaning methods to eradicate these biofilms. The environmental conditions in meat processing facilities, typically cold and damp, further complicate sanitation efforts and can diminish the efficacy of disinfectants and sanitizers, adding another layer of challenge to maintaining hygiene standards. In this abstract, we are presenting initial investigation of UVC fluorescence imaging system to assess facility hygiene as case study for the first time without a scientific methodology to prove a hypothesis.

II. MATERIALS AND METHODS

Fluorescence imaging has been successfully utilized to detect contamination on meat carcasses [1], Despite its success in carcass inspection, fluorescence imaging has not yet been reported as a tool for inspecting hygienic environmental surfaces. In this study, we evaluated a handheld fluorescence imaging system, Figure 1(a), that utilizes UVC LEDs for excitation at 275 nm and emission at 350 nm. This system specifically targets amino acids—components found in nearly all proteins—which serve as effective markers for detecting protein residues on surfaces using UVC fluorescence imaging. The handheld scanner, named Contamination Sanitization Inspection and Disinfection (CSI-D+), employs pulsed UVC LEDs to illuminate contaminated surfaces. A UV-sensitive CMOS camera, synchronized with the LED pulses, captures both fluorescence and ambient light. The fluorescence images are then derived by subtracting the image taken with the LED turned off from the image taken with the LED turned on. This process allows the operator to detect contamination on surfaces in bright ambient light conditions.

III. RESULTS AND DISCUSSION

Two cases utilizing the CSI-D+ system to identify contaminated surfaces with 275 nm excitation illustrate the system's efficacy. In the first case, a rack of cutting knives that had already undergone a cleaning procedure was inspected. As shown in Figure 1(b), there were no visible signs of contamination on the knife handle.

However, Figure 1(c) reveals that the CSI-D+ system detected contamination not visible to the naked eye. Consequently, the specific knife was subjected to a second cleaning procedure. A follow-up inspection using the CSI-D+ system validated the knife's complete cleanliness as shown in Figure 1(d). In the second case, a meat scale was inspected before and after cleaning. Initially, the surface had not been cleaned as shown in Figure 1(e), and the inspection ensured that the CSI-D+ system's readings, Figure 1(f), correlated with the visual contamination observed. After the cleaning procedure, the meat scale was scanned again, and Figure 1(g) shows the verification of the cleaned surface, confirming the system's accuracy in detecting and validating cleanliness.

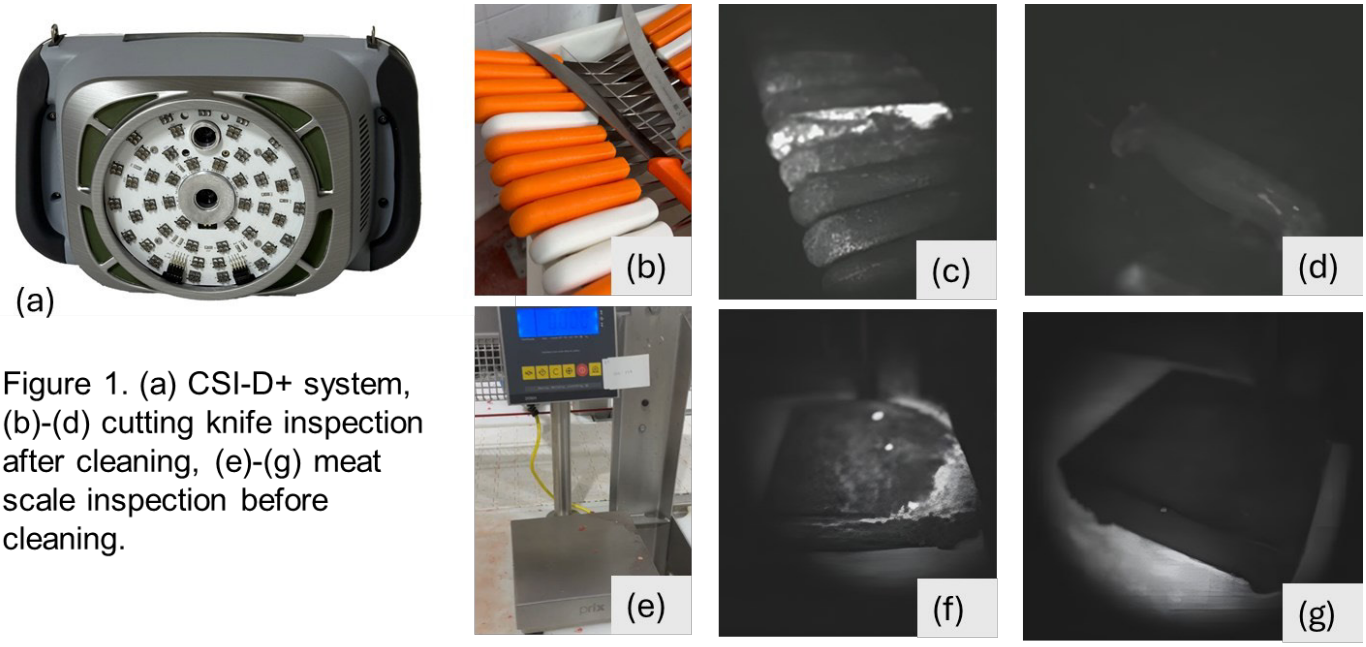

Figure 1. (a) CSI-D+ system, (b)-(d) cutting knife inspection after cleaning, (e)-(g) meat scale inspection before cleaning.

IV. CONCLUSION

In conclusion, the study demonstrated the effectiveness of the handheld fluorescence imaging system (CSI-D+) in identifying protein residues on surfaces within meat processing facilities. The use of UVC LEDs for excitation and a UV-sensitive CMOS camera for capturing fluorescence images provides a rapid and efficient method for detecting contamination. This approach addresses the limitations of traditional microbial testing methods, which are often slow and incapable of real-time monitoring. The CSI-D+ system's ability to reveal biofilms and protein residues that are invisible to the naked eye offers a significant advancement in maintaining hygiene standards and preventing cross-contamination. By implementing such technology, meat processing facilities can improve their cleaning protocols, ensuring adherence to hygiene regulations and reducing the risk of foodborne pathogens. This study underscores the potential of fluorescence imaging as a vital tool in the continuous effort to enhance food safety and sanitation practices in the meat processing industry.

The combination of fluorescence imaging and machine learning techniques offers significant benefits, particularly in training models to identify and segment contamination consistently. By training fluorescence images with known contamination data, a segmentation model similar to the one described in previous studies can be developed. This model allows users with varying skill levels to operate the scanner and obtain reliable, consistent results.

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Profiling of microbial populations as tool for meat quality and safety

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I. INTRODUCTION

Microbiological testing is commonly employed to verify hygienic conditions during slaughtering, as well as downstream operations in meat transformation, and to help in the management of food safety. Commonly, testing is focused on select microbial groups (indicator microorganisms) or target pathogens. International and national guidelines aid producers in setting internal standards regarding load/concentration of indicator/pathogenic microorganisms within the context of food safety management systems.

Microbiological testing has in recent times expanded its scope thanks to the introduction of Next Generation Sequencing (NGS) applications. Amplicon sequencing is employed to get insight into the ecology, in terms of taxonomy, of complex microbial communities, while metagenome sequencing is used when information regarding potential functions of the microbial communities is desired. Such methodologies are applied both for food and environmental samples and are rapidly expanding in various sectors of the food industry, including the meat sector.

This paper provides examples of potential applications of NGS, coupled with classic microbiological testing, in the meat sector.

II. MATERIALS AND METHODS

Meat and environmental samples (from processing facilities) were collected using standard procedures. Samples were transferred in the lab under refrigerated conditions and within 2 hours from collection. Classic microbiological analyses were immediately performed while aliquots for nucleic acid (DNA or RNA) extraction were in parallel collected and appropriately conserved. Nucleic acid extraction was performed using commercial kits and 16S rRNA encoding gene amplicon sequencing (V3-V4 region) was performed. Sequencing data were treated using the QIIME platform and statistical analysis was performed by R. For more detailed information on materials and methods refer to Botta et al. 2020 [1] and Botta et al. 2023 [2].

III. RESULTS AND DISCUSSION

Figure 1 depicts the shift in the microbial communities before and after standard cleaning and sanitation procedures in a commercial beef slaughterhouse facility. As can be seen, the microbial communities residing on surfaces of the slaughterhouse before and after cleaning/sanitation are distinct. In this case, amplicon sequencing provided a global overview of the microbiota and how it is influenced by common activities in the facility.

Figure 2a shows the effect of ozone treatment, applied following standard cleaning and sanitation procedures, on the microbial communities of surfaces in a beef slaughterhouse facility. Ozone treatment (12 hours of gaseous treatment at 20 ppm) profoundly and differentially influenced certain taxa. *Carnobacterium*, *Pseudomonas* and *Brochothrix* significantly decreased while *Staphylococcus aureus* showed an increase although not significant. Importantly, the trends identified through sequencing could be confirmed by viable counts, as shown in Figure 2b. Metaxonomic evaluation clearly indicated what could be the outcome in terms of modification of the residing microbiota when innovative microbiostatic/microbiocidal interventions are applied in a processing plant.

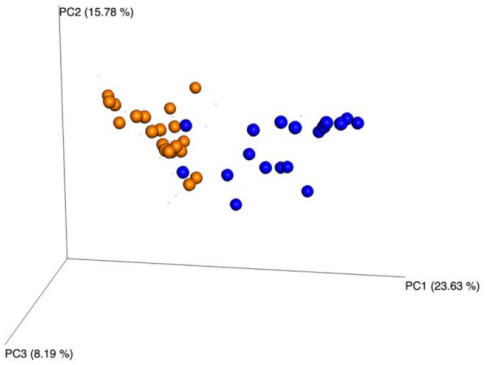

Figure 1. PCoA chart displaying the Weighted UniFrac distance matrix (β-diversity) of a slaughterhouse environment before (blue) and after (orange) cleaning-sanitizing procedures; BC and ACS are different communities (P < 0.001 [FDR adjusted]; ANOSIM and ADONIS tests) [1].

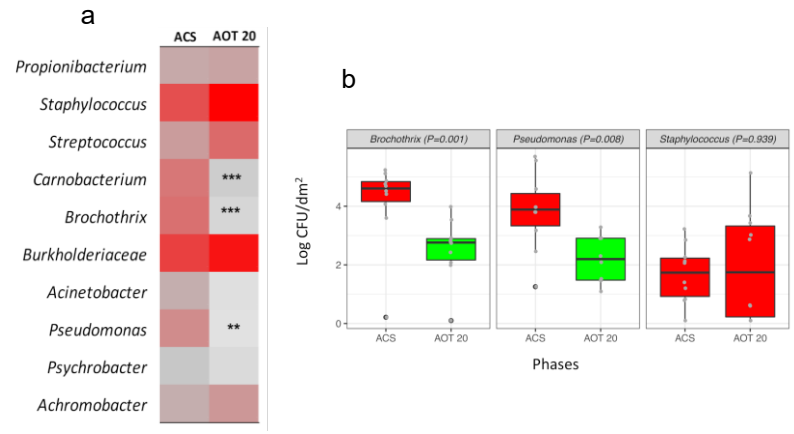

Figure 2. Pseudo-heatmap (a) summarizing the abundance variations of the 10 core OTUs that occurred during the ozone treatments; asterisks highlight significance decrease of relative abundance. Viable counts (**b**) of *Brochothrix*, *Pseudomonas* and *Staphylococcus* before (ACS) and after (AOT 20) a 20 ppm ozonation. Box-plot colors highlight significant differences between ACS and AOT 20 counts. Modified from [1].

When a metataxonomic approach was employed to explore the microbiota of individual carcasses at slaughtering, it was observed that the microbial communities on the carcass surface varied depending on the origin (animal) (Figure 3a). In addition, a comparison was made between the taxa detected on the carcasses before and after cooling. A higher number of unique taxa was observed following a cooling phase (24 hours at 2-4 °C) (Figure 3b) and was mainly represented by psychrotrophs (data not shown).

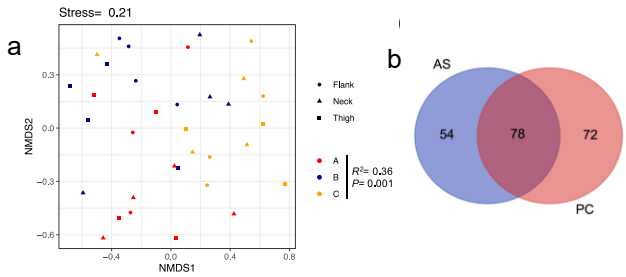

Figure 3. Biplot (**a**) of the Non-Multidimensional Scaling (NMDS) analysis coloured by animal origin, which significantly discriminated the samples. Venn diagrams (**b**) displaying the shared taxa between animals (A, B, C), temporal phases (After slaughtering, AS and post cooling, PC) and sampling areas (Neck, Flank, Thigh). Modified from [2].

IV. CONCLUSION

The examples here presented highlight the depth of information that can be obtained through the application of NGS methodologies in food microbiology. It is now possible to consider food and environmental samples as small ecosystems and through a microbial ecology perspective, study in detail the interactions among microbial groups and how they may be influenced by intrinsic and extrinsic factors that are presented along the production chain.

ACKNOWLEDGEMENTS

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SESSION 7

Objective measurement of carcass and meat quality

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EFFECTS OF GRADED INCLUSION LEVELS OF SORGHUM IN FINISHER DIETS FOR STEERS ON BEEF FATTY ACID PROFILES

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I. INTRODUCTION

Coupled with the increase in the human population and the emergence of health-conscious consumers, meat production is constrained by climate-induced feed scarcity and price spikes. For example, the average global price for maize grain, the main energy source in livestock diets, rose by more than 50% from 2020 to 2023 [1]. This calls for a paradigm shift towards the use of climate-resilient feed resources. In this context, sorghum stands out for its climate resilience, and comparable nutritional composition and animal performance to maize [2]. More interestingly, sorghum has higher contents of polyphenols (0.25 – 11.5 g GAE/ kg DM) [3] and proportions of alpha-linolenic acid (ALA, C18:3n-3; 0.6 – 5% of total fatty acids, TFA [4]) than maize. Sorghum also has comparable proportions of linoleic (LA, C18:2n-6; 27 – 52% of TFA) and oleic (C18:1n-9; 30 – 50% of TFA) acids to maize (ALA, 0.6 – 1%; LA, 38 – 57%; oleic, 31 – 33% of TFA), respectively [4,5]. Sorghum polyphenols could protect dietary polyunsaturated fatty acids (PUFA) from rumen biohydrogenation (BH) and modify the rumen environment to favour the production of health-enhancing BH products such as rumenic acid and its processor vaccenic acid which will be absorbed in the small intestines and deposited in the muscle [6,7]. However, little, if any information, is known about the effect of feeding cattle sorghum-containing finisher diets on beef fatty acid (FA) composition. Thus, the FA composition of beef from steers-fed graded levels of sorghum as a replacement for maize was evaluated in the current study.

II. MATERIALS AND METHODS

Thirty-five Angus steers (n = 7) were randomly assigned to five finisher diets containing either 0, 100, 200, 300 or 400 g/kg DM of sorghum substituting white maize. The steers were slaughtered after a 90-day feeding trial preceding a 21d adaptation period. After 24 h postmortem, the left *Longissimus thoracis et lumborum* (LTL) for each animal was harvested from 9th to 13th rib for FA analysis. The lipid was extracted using chloro-methanol extraction, methylated with two-stage acid-base protocol and FAMEs analysed using a GC with a 100 m capillary column and a 175 °C temperature program. All the fatty acid data was handled with GLIMMIX procedure of SAS including diet as a fixed factor.

III. RESULTS AND DISCUSSION

Increased substitution of sorghum for maize in beef finisher diets did not affect ($P > 0.05$) beef fatty acid composition (Table 1). The lack of difference in the fatty acid profile of beef in the current study could be attributed to a slightly similar dietary fatty acid profile and low phenolic contents. The dietary polyphenols observed were below 20 g/kg DM known to influence rumen biohydrogenation and lipolysis [6,7].

Table 1: Profile of selected fatty acids (mg/100 g) of beef *Longissimus thoracis et lumborum* from steers fed finisher diets containing sorghum substituted for maize

	Sorghum inclusion (g/kg DM) in the diet					SEM[1]	*P*-value
Variable	**0**	**100**	**200**	**300**	**400**		**Diet**
∑Total fatty acid methyl esters	2114.2	2297.7	2267.7	2195.4	2241.6	247.23	0.987
∑Polyunsaturated fatty acids	118.2	115.5	113.9	112.0	111.2	10.22	0.990
∑*n*-6 Polyunsaturated fatty acids	90.4	88.1	88.2	85.7	84.6	9.33	0.993
18:2*n*-6	64.8	63.9	64.8	62.2	61.6	7.40	0.997
18:3*n*-6	2.2	2.3	2.3	2.2	2.2	0.20	0.993
20:3*n*-6	2.5	2.6	2.4	2.4	2.4	0.33	0.992
20:4*n*-6	19.5	17.9	17.6	17.6	17.2	2.70	0.981
22:4*n*-6	1.2	1.2	0.9	0.9	0.9	0.16	0.417
∑*n*-3 Polyunsaturated fatty acids	14.5	14.6	14.1	14.6	14.9	1.35	0.994
18:3*n*-3	10.5	11.0	10.3	10.7	10.9	1.26	0.995
22:5*n*-3	4.0	3.6	3.8	4.0	4.1	0.63	0.987
∑Conjugated linoleic acid	13.3	12.8	11.6	11.7	11.6	0.94	0.590
*c*9,*t*11-18:2	7.1	7.1	6.0	5.9	6.0	0.59	0.352
*t*10,*c*12-18:2	3.1	2.9	2.9	3.0	3.1	0.45	0.995
*c*11,*t*13-18:2	2.4	2.4	2.4	2.4	2.2	0.38	0.997
*t*9,*c*12-18:2	0.5	0.4	0.4	0.4	0.4	0.09	0.107
∑Monounsaturated fatty acids	1114.3	1175.9	1145.3	1169.2	1172.6	149.07	0.998
*c*9-16:1	70.2	72.1	71.4	72.6	72.2	10.75	0.999
*t*10/*t*11-18:1	40.2	38.3	40.1	41.9	42.6	6.41	0.991
*c*6-18:1	69.5	71.8	69.2	71.5	72.9	7.86	0.997
*c*9-18:1	896.0	954.5	926.2	946.3	947.3	137.62	0.998
∑Saturated fatty acids	881.8	1006.3	1008.5	914.2	957.8	117.39	0.921
12:0	2.2	3.0	2.5	2.7	3.1	0.41	0.508
16:0	545.1	658.9	652.8	557.8	566.4	83.80	0.785
18:0	221.5	218.0	228.2	236.0	265.1	26.50	0.732

∑: Summation; SEM: Standard error of means.

IV. CONCLUSIONS

Replacing maize with sorghum in finisher diets of steers had neutral effects on the health value of meat.

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Aging Methods Effect on Meat Quality Attributes from Steers Under Different Finishing Diets

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I. INTRODUCTION

Aging is a post-mortem practice for beef tenderization and flavor improvement. Wet and dry aging are the most common processes to age beef. Dry aging in a highly moisture-permeable bag has been widely used in the last decades, producing dry-aged products mainly to reduce microbial contamination, lipid oxidation, and trim loss when compared to the traditional out-of-bag dry-aging technique [1]. On the other hand, finishing diet has been an important factor affecting beef- quality attributes. Meat from grain- and pasture-fed animals presents different physical, organoleptic, and nutritional characteristics [2]. The study evaluates the effects of different and combined aging methods from pasture-fed and grain-fed steers on meat quality attributes.

II. MATERIALS AND METHODS

This study involved 60 paired striploins from British breed steers under 30 months of age, finished (F) on pasture (15) or grain (15). Striploins were assigned to different aging methods (AM) for 40 days, including dry aging in a bag (DAb-40 d), wet aging (WA-40 d), and their combinations (DW: DAb-20 d + WA-20 d and WD: WA-20 d + DAb-20 d). The lean surface color was measured using a Minolta Chroma Meter CR-400. Warner Bratzler Shear Force (WBSF) was evaluated on six cores per steak using a TA-XT Plus texture analyzer (Stable Micro System Ltd., UK). Intramuscular fat content (IMF) was assessed using the lipid extraction method, followed by the analysis of the fatty acid (FA) composition described by Correa et al. [3]. Lipid oxidation was determined using the TBARS method, with the methodology described by Correa et al. [3]. The experimental design was a split-plot and the statistical analysis was performed with a model including the fixed effects of F and AM, their interaction, and the random effect of the carcass using the MIXED procedure (v. 9.4, SAS Institute Inc., Cary, NC, US). The significance level was set at $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

Results are presented in Table 1. Higher L^* values found in WA may be explained by a greater reflectance associated with more moisture. However, a^* and b^* coordinates presented a significant ($P<0.05$) interaction AM * F where the highest ($P<0.05$) values of a^* were in WA regardless of the F, WD from pasture-fed animals, and DAb from grain-fed steers. This result might be attributed to the greater water content from the WA process, so more water on the meat's surface results in lighter red [4]. Also, DAb from grain-fed steers showed greater ($P<0.05$) b^* values than the other treatments. Apaoblaza et al. [5], in agreement with our study, reported lower L^* values on meat from forage-fed animals than grain-fed cattle, which would be associated with an increased myoglobin content (more muscle activity) making them darker. Consistent with other experiments [1], our study showed no differences ($P>0.05$) in WBSF between aging methods, and the values were lower than 3 kgF, below the threshold for consumer acceptance. After the aging period, DW showed a greater IMF than WA meat ($P<0.05$). In agreement with previous studies [2], the composition of FA groups did not differ between AM. However, a significant ($P<0.05$) AM*F interaction was observed for FA composition and the TBARS values. The highest ($P<0.05$) concentrations of saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA), and TBARS values were found in WD from grain-fed steers. In contrast, polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (CLA) concentrations were greater

(*P*<0.05) in the AM from pasture-fed than from grain-fed steers, except for PUFA in WA and DW. The *n-6:n-3* fatty acids ratio was ≤4 in meat from pasture-fed animals in agreement with the recommended intakes of FAs performed by the Department of Health (1994) of the United Kingdom. The PUFA/SFA ratio was greater in the IMF of meat from pasture-fed than grain-fed steers. The results of the FA in the current study are aligned with previous findings reported [2].

Table 1. Effects (mean ± SEM) of aging method (AM) and finishing diet (F) and their interaction (A*F) on physicochemical traits.

	Aging (AM)				Finishing (F)		A*F
Traits	DAb	WA	DW	WD	Pasture	Grain	*P-value*
L*	**40.5±0.4b**	**41.8±0.4a**	**40.9±0.4ab**	**41.3±0.4ab**	**40.0±0.4b**	**42.2±0.4a**	*0.350*
a*	**22.2±0.4b**	**24.0±0.4a**	**21.7±0.4b**	**22.3±0.4b**	22.4±0.4	22.7±0.4	***0.011***
b*	**11.8±0.2a**	**11.9±0.2a**	**10.7±0.2b**	**11.1±0.2b**	11.3±0.3	11.4±0.3	***0.029***
WBSF (kgF)	2.6±1.0	2.5±1.0	2.6±1.0	2.5±1.0	2.7±1.2	2.5±1.2	*0.198*
IMF (%)	**3.9±0.2ab**	**3.7±0.2b**	**4.2±0.2a**	**4.1±0.2ab**	**3.7±0.2a**	**4.2±0.2b**	*0.623*
CLA (mg/100g)	19.8±1.6	20.9±1.7	23.4±1.9	24.9±2.0	**28.7±2.4a**	**17.0±1.4b**	***0.008***
SFA (mg/100g)	2043.0±144.0	2019.4±142.3	2186.8±154.1	2205.1±157.7	2071.5±142.8	2153.2±149.0	**0.018**
MUFA (mg/100g)	1780.4±126.6	1732.4±123.1	1961.7±139.4	1970.6±142.1	1686.5±117.5	2047.5±143.1	**0.026**
PUFA (mg/100g)	251.4±14.4	238.2±13.6	238.5±13.7	251.3±14.6	**277.6±11.3a**	**217.3±9.8b**	**0.045**
n6:n3	3.1±0.17	2.9±0.16	3.1±0.17	3.0±0.17	**2.1±0.15b**	**4.5±0.31a**	0.336
PUFA/SFA	0.12±0.007	0.12±0.007	0.11±0.007	0.12±0.007	**0.14±0.01a**	**0.10±0.008b**	0.083
TBARs (mg/kg)	**0.37±0.03ba**	**0.34±0.02b**	**0.43±0.02a**	**0.42±0.03a**	**0.32±0.02b**	**0.47±0.03a**	**0.010**

DAb: Dry aging bag; WA: Wet aging; DW: Dry aging bag 20d + WA 20d; WD: Wet aging 20d + Dry aging bag 20d. WBSF: Warner Braztler Shear Force; IMF: intramuscular fat; CLA: conjugated linoleic acid; SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: polyunsaturated fatty acid; *n6:n3*: PUFA-n6/PUFA-n3; TBARS: Thiobarbituric acid-reactive substances (mg MDA/kg meat). LS means with different letters in the same row denotes significant differences (*P*<0.05).

IV. CONCLUSION

Although all the aging methods showed at least acceptable performance concerning meat quality, the combination of both aging techniques provides no benefit when compared to a single aging process. The dry bag and wet-aging process alone for meat from grain-fed steers appear to be valuable regarding its technical and nutritional quality. Further research is warranted to identify optimal combinations of dry bag-aging/wet-aging times to develop an in-depth understanding of the safety and quality of extended aging and stepwise aging in fresh beef.

ACKNOWLEDGEMENTS

The authors would like to thank INIA Uruguay for the funding that made this study possible.

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TREATMENT OF LAMB MEAT WITH HIGH-INTENSITY ULTRASOUND

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I. INTRODUCTION

The evolution of food processes is driven by changes in consumer preferences and the need to produce safe, high-quality food [1]. Ultrasound is an acoustic energy [2], therefore, it is a non-ionizing, non-invasive and non-polluting form of mechanical energy [3]. It is considered an emerging method with great potential to control, improve and accelerate processes without compromising food quality [4].

The aim of this study was to evaluate the qualitative characteristics such as pH, color, water holding capacity, cooking loss and tenderness of lamb meat subjected to high-intensity ultrasound treatment.

II. MATERIALS AND METHODS

The study was approved by the ethics committee for the use of animals in research at the Federal University of Paraná (protocol number 050/2023).

Six loins and six flat were used from the carcass of 120-day-old lambs that were finished in confinement and slaughtered with 38 ± 2 kg of body weight. After being removed from the carcass, the cuts were cleaned to remove subcutaneous fat and connective tissue and were vacuum packed and frozen (- 18°C) until analysis was carried out. Subsequently, the cuts thawed in a BOD incubator for 12 hours at 4°C and then subjected to three times in the ultrasound bath: time zero (without ultrasound bath), 5 and 10 minutes. The ultrasound bath (usc-2800a, Unique) used worked at a frequency of 40kHz, temperature of 25°C and a power of 100 W, representing high intensity ultrasound (10–1000 Wcm^{-2}) and low frequency (20–100kHz).

After the ultrasound bath were analyzed: pH, color, water holding capacity (WHC), cooking loss (CL) and shear force (SF). The pH was measured with a peagameter (Testo), and the color was measured on the surface of the samples after being exposed to the environment for 30 minutes. To measure the coordinates L* (lightness), a* (redness) and b* (yellowness), a Minolta CR-400 colorimeter was used. To obtain the WHC, 0.5 g of meat sample was weighed, placed inside a filter paper and between two acrylic plates and a 10 kg weight was placed on the plates for 5 minutes, after the sample was weighed again and the WHC was given as a percentage of the initial weight. To measure the CL, a 5 x 5 cm sample from each cut was weighed and placed to bake on a grill until the internal temperature reached 70°C. Later, after the samples had cooled and reached a constant weight, they were weighed again and the CL was obtained by difference between the initial weight and the final weight, divided by the initial weight, expressed as a percentage. The cooked samples were cut into cylinders (3 cylinders by sample) and subjected to cutting in the transverse direction of the muscle fibers using the Texture Analyzer device, coupled to the Warner-Bratzler blade, with the values expressed in kgf.

The design was completely randomized in a 2 x 3 factorial scheme, two cuts (loin and flat) and three ultrasound times (0, 5 or 10 minutes). The means were compared using the Tukey test at 5% significance and the Minitab 18.0® program was used for statistical analysis.

III. RESULTS AND DISCUSSION

An interaction between cut and ultrasound time (P= 0.008) was observed only for b*, which was higher in the flat with 11.6 and 12.5 when compared to loin with 9.7 and 9.3 at times 0 and 5 minutes of

ultrasound bath, respectively. In 10 minutes of ultrasound the value of b* was 12.3 for loin and 11.9 for flat. The interaction was not observed (P≥ 0.05) for the other parameters evaluated (Table 1).
The flat had a lower a* (P= 0.001) when compared to the loin, 7.9 and 8.8, respectively. Studies carried out comparing the color of the Longissimus lumborum and Biceps femoris muscles did not observe a difference in the a* content between them, however the greater amount of connective tissue in the Biceps femoris can change the a* value, although it is lighter. Even though the value of L* at time 5 did not differ from the others, it appears that the L* content increased with ultrasound time, the value of L* at time 0 was 45.5, at time 5 it was 49.0 and at time 10 was 51.0 (Table 1). According to Stadnik and Dolatowski (2011), ultrasound accelerates total changes in color, limits the formation of oxymyoglobin, and slows down the formation of metmyoglobin. However, effects of ultrasound treatment on the color of fresh meat are not widely reported. Still, as in the present study, some others [5] found changes in the color of fresh meat after ultrasound treatment, becoming less shiny, less red and more yellowish.

Table 1 – Qualitative traits mean (± standard error) of lamb meat (loin and flat) subjected to high-intensity ultrasound treatment for 0, 5 or 10 minutes.

Trait	Cut			Ultrasound times (minutes)				Pr> F interaction
	Loin	Flat	Pr>F	0	5	10	Pr>F	
pH	5.7 (±0.03)	5.7 (±0.05)	0.491	5.6 (±0.07)	5.7 (±0.04)	5.7 (±0.06)	0.936	0.380
L*	47.0 (±0.91)	50.0 (±1.23)	0.079	45.5b (±0.60)	49.0ab (±1.24)	51.0a (±1.06)	0.012	0.501
a*	8.8 (±0.01)	7.9 (±0.15)	0.001	8.4 (±0.16)	8.3 (±0.31)	8.3 (±0.36)	0.947	0.099
WHC (%)	63.2 (±2.53)	62.2 (±1.45)	0.734	62.6 (±2.32)	58.1 (±1.52)	66.7 (±1.85)	0.051	0.206
CL (%)	24.8 (±1.40)	31.4 (± 1.51)	0.017	30.1 (±2.56)	26.1 (±2.29)	27.9 (±2.54)	0.424	0.959
SF (kgf)	3.7 (±0.34)	2.7 (±0.14)	0.021	3.2 (±0.33)	3.5 (±0.53)	2.8 (±0.36)	0.524	0.886

L* (lightness) and a* (redness); WHC= water holding capacity; CL= cooking loss; SF shear force.
For ultrasound time, the means with different letters in the line differ statistically using the Tukey test at 5% probability.

The flat was tender than loin and had greater CL (Table 1), the opposite was expected, as the Biceps femoris muscle, being in the leg, tends to be harder than Longissimus lumborum. The low age of the animals at slaughter (120-day-old) and finishing in confinement may explain the low SF of flat. However, for both cuts the values of SF are ideal for lamb meat (Table 1). Cut and ultrasound time did not influence (P≥ 0.05) pH and WHC values (Table 1).

IV. CONCLUSION

The treatment of lamb meat with high-intensity ultrasound negatively affected the color, increasing the lightness and the yellowness. More studies are needed evaluating the use of this technology in sheep meat.

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CARCASS AND MEAT QUALITY ATTRIBUTES OF FOUR PUREBRED ZEBU YOUNG BULLS

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I. INTRODUCTION

Meat production in Brazil relies on *Bos taurus indicus* (Zebu) animals due to their high rusticity and resistance to parasites. A portion of this production comes from purebred animals, which have information proving their genetic lineage. Research on these animals is crucial for standardization of meat quality, adding commercial value, and selecting desirable characteristics for genetic improvement. Thus, the objective of this study was to compare the carcass and meat quality of young bulls from four purebred Zebu breeds.

II. MATERIALS AND METHODS

Purebred uncastrated young bulls (n = 110) from four breeds, Brahman (n = 17), Guzerá (n = 25), Sindi (n = 23) and Tabapuã (n = 41), were kept under the same conditions since wean. Animals were fed on pasture for 10 months supplemented with mineral salt, and finished for 120 days in feedlod. After weighing the animals and slaughtering, carcass attributes (dentition, carcass weight, fat cover, marbling, loin eye area, and backfat thickness) were evaluated. After carcass cooling, a ~20 cm portion of the striploin (*Longissimus lumborum*) were collected after 48 hours of slaughter. Samples were vacuum-aged for 14 days for meat quality evaluation: pH; moisture, total lipid, and total protein (AOAC, 2007); cooking loss and shear force (AMSA, 2015).

The fat cover and marbling data were evaluated by descriptive analysis. The other data was analyzed using Statistica 10.0 software, using Analysis of Variance and the Tukey test (5%); Pearson's correlation was also evaluated.

III. RESULTS AND DISCUSSION

All animals were under 24 months, with zero permanent incisor teeth. The fat cover was mostly similar for all breeds. The degree of marbling was low for all breeds, with a predominance of Practically Devoid, followed by Traces (Figure 1).

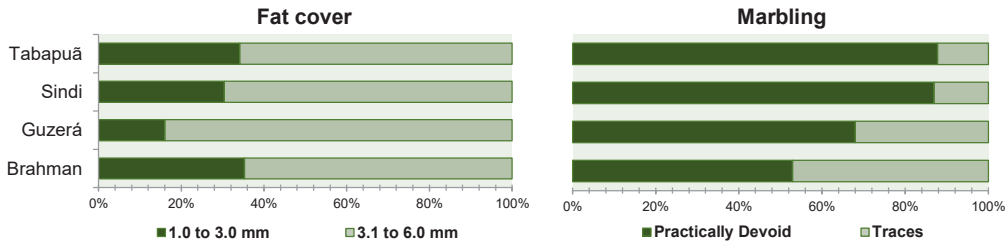

Figure 1. Fat thickness and degree of marbling in Zebu bulls.

Despite the animals being subjected the same handling conditions, the performance of the Brahman, Guzerá and Tabapuã breeds was superior to that of Sindi, both in terms of live weight and carcass weight ($P < 0.05$) (Table 1).

Loin eye area was higher in the Brahman and Tabapuã breeds, with the Brahman differing from the Guzerá and Sindi. When carcass weight was adjusted to 300 kg, the adjusted loin eye area was higher for the Brahman and Sindi breeds, and lower for the Guzerá and Tabapuã ($P < 0.05$). Regards to backfat thickness, Tabapuã showed the greater thickness, differing only from the Guzerá (Table 1).

There were no differences between the breeds for pH, moisture, and total lipid (P > 0.05). The total protein was significantly lower for the Sindi breed compared to the other breeds (Table 1).

Table 1 – Carcass and meat quality attributes of Zebu young bulls.

Parameters	Brahman (n=17)	Guzerá (n=25)	Sindi (n=23)	Tabapuã (n=41)	*P*-value
Live weight, kg	628.86 ± 11.53 [a]	602.72 ± 9.85 [a]	508.64 ± 11.21 [b]	622.91 ± 8.34 [a]	<0.001
Carcass weight, kg	342.1 ± 6.70 [a]	333.4 ± 5.94 [a]	285.8 ± 7.25 [b]	353.9 ± 5.35 [a]	<0.001
LEA, cm^2	85.35 ± 3.23 [a]	75.34 ± 1.43 [b]	73.28 ± 1.48 [b]	79.33 ± 1.43 [a,b]	<0.001
Adjusted LEA*, cm^2	74.67 ± 1.99 [a]	67.91 ± 0.90 [b]	77.44 ± 1.45 [a]	67.44 ± 1.02 [b]	<0.001
Backfat thickness, mm	3.79 ± 0.40 [a,b]	3.28 ± 0.22 [b]	3.63 ± 0.43 [a,b]	4.45 ± 0.28 [a]	<0.05
pH	5.59 ± 0.02 [a]	5.61 ± 0.01 [a]	5.59 ± 0.01 [a]	5.58 ± 0.01 [a]	0.58
Moisture, %	72.82 ± 0.20 [a]	72.91 ± 0.16 [a]	73.20 ± 0.14 [a]	73.13 ± 0.09 [a]	0.24
Total lipid, %	2.65 ± 0.26 [a]	2.63 ± 0.13 [a]	2.32 ± 0.12 [a]	2.53 ± 0.06 [a]	0.29
Total protein, %	24.22 ± 0.20 [a]	23.94 ± 0.14 [a]	23.16 ± 0.16 [b]	23.70 ± 0.11 [a]	<0.001
Cooking loss, %	20.94 ± 0.42 [a]	21.11 ± 0.44 [a]	20.66 ± 0.33 [a]	20.33 ± 0.26 [a]	0.33
Shear force, kg	4.13 ± 0.19 [b]	4.47 ± 0.22 [a,b]	5.17 ± 0.28 [a]	4.51 ± 0.16 [a,b]	<0.05

[a,b] Means (± standard error of mean) with different letters in the same row differ from each other by analysis of variance (P <0.05). LEA: Loin eye area. *LEA ÷ carcass weight × 300.

Cooking loss was not affected by breed (P > 0.05), but the Sindi breed had the highest average of WBSF value compared to the Brahman breed (P < 0.05) (Table 1). According to the classification proposed by ASTM (2011), samples from Brahman animals are considered tender (with a range of 4.0 to 4.4 kg), while the other breeds were classified as tough (WBSF > 4.5 kg). These characteristics are crucial when making decisions about introducing a particular breed into a meat brand.

Negative correlations were observed between shear force with live weight (r = -0.25; P < 0.05), carcass weight (r = -0.19; P < 0.05), backfat thickness (r = -0.23; P < 0.05), and with total intramuscular lipid (r = -0.33; P < 0.001). Heavier and fatter carcasses tend to decrease the speed of carcass chilling, avoiding the occurrence of cold shortening. In the case of intramuscular fat, higher values are related to tender meat. It may explain the negative correlations with instrumental tenderness (Miller, 2024).

IV. CONCLUSION

The four breeds exhibited variations in carcass and meat quality metrics. Brahman bulls demonstrated superior growth performance, displaying the highest animal weight and carcass weight, alongside the higher loin eye area and backfat thickness. Moreover, the meat from these animals exhibited enhanced quality, characterized by a tender texture compared to other breeds.

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DOES REARING SYSTEM IMPACT ON METABOLITE COMPOSITION OF DAIRY LAMB MEAT AS MEASURED USING RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY?

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I. INTRODUCTION

The dairy sheep industry is small but a growing part of the New Zealand agricultural sector [1]. Lambs from dairy sheep farming systems are either naturally reared (NR) through provisioning of maternal milk or artificially reared (AR) through feeding milk replacement formula, and weaned off milk from 4 to 6 weeks of age. Our previous study observed minor impacts of rearing systems on fatty acid profile and consumer liking of lamb meat [2], while it remains unknown whether two rearing methods would alter the metabolite profile of lamb meat. Rapid Evaporative Ionization Mass Spectrometry (REIMS) is an emerging tool for rapid authentication of muscles and meat quality assessment based on metabolite fingerprints [3]. This study aimed to determine the effects of rearing methods on metabolite changes, measured by REIMS, in two muscles from dairy lambs.

II. MATERIALS AND METHODS

A subset of twin-born East-Friesian-cross dairy lambs from a larger cohort study (n=96) were slaughtered at 18 weeks of age following natural rearing on the dam (NR; n=19) on pasture as twins or indoor artificial rearing (AR; n=19) on ad libitum milk replacer, as described in previous study by Pavan et al. [2]. All lambs were weaned off milk at 6 weeks of age and were managed on pasture thereafter. All lambs received a grain-based concentrate from 3 to 12 weeks of age and were finished on the same pasture. Lamb *m. longissimus thoracis* (LT) and *m. semitendinosus* (ST) muscles were collected from the same side of the animal at 24 h postmortem. Sub-samples were taken from the cranial end of both muscles and frozen at -80 °C for REIMS analysis. Lamb samples were thawed at 4 °C overnight and their metabolite features were determined in triplicate by the laser-assisted-REIMS (Waters, Wilmslow, UK) coupled to a quadrupole-time of flight (qToF) mass spectrometer (Waters Xevo® G2 qToF, Waters). The mass spectra were collected between *m/z* 500 and 1200 at 0.5 Hz in negative ionization mode with measurement times of <10 seconds/sample. Mass spectral features were mass corrected, aligned and library matched against the Human Metabolome Database and LipidMaps database. The mass features were analyzed by PCA (Principal Component Analysis), OPLS-DA (Orthogonal Projection to Latent Structures-Discriminant Analysis) and evaluated by ROC (Receiver Operating Characteristics) curves (SIMCA 16, Umetrics, Sweden). R^2 (cumulative) and Q^2 (cumulative) scores were generated to evaluate the robustness and accuracy of the OPLS-DA models. Individual features were considered different at $P_{adjr}<0.05$ based on t-tests adjusted for Benjamini-Hochberg false discovery rate.

III. RESULTS AND DISCUSSION

A total of 2,017 features were detected, of which 696 had tentative identifications based on library matching of high-resolution mass (mass error <5 ppm) after data cleanup (QC variation <30%). These features were used for further data analysis. The metabolite fingerprints differed between the two

rearing systems (OPLS-DA model: Q^2=0.56, R^2=0.63; AUC=0.998, Figure 1), suggesting REIMS can accurately discriminate lamb samples from AR and NR. However, absolute differences in metabolites were limited to 4 features (P_{adj}<0.05) tentatively annotated as 9-hexadecen-1-ol, octadec-11Z-enol, PA(P-16:0/0:0), and PE(P-16:0/20:5), which agreed with our previous observation that rearing systems played a minimal role in affecting the nutritional composition and quality of dairy lamb meat [1]. OPLS-DA (Q^2=0.77, R^2=0.80) and feature-reduced PCA models were more readily distinguished between muscle types, showing that the metabolite fingerprints of the two lamb muscles differed (Figure 2) regardless of the rearing system. LT and ST muscles can be accurately discriminated (AUC=0.94) by REIMS with 99 features (P_{adj}<0.05) driving the separation, of which the ions with the lowest P-values were mainly identified as phospholipids.

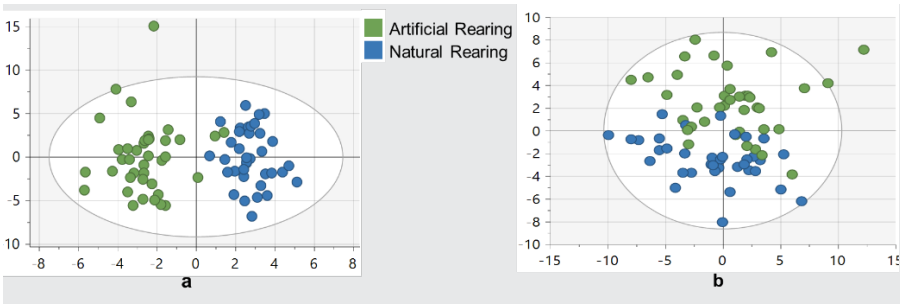

Figure 1. OPLS-DA (a) and feature-reduced PCA (b) score plots of lamb muscles from artificial rearing (AR) compared to natural rearing (NR) regardless of muscle type.

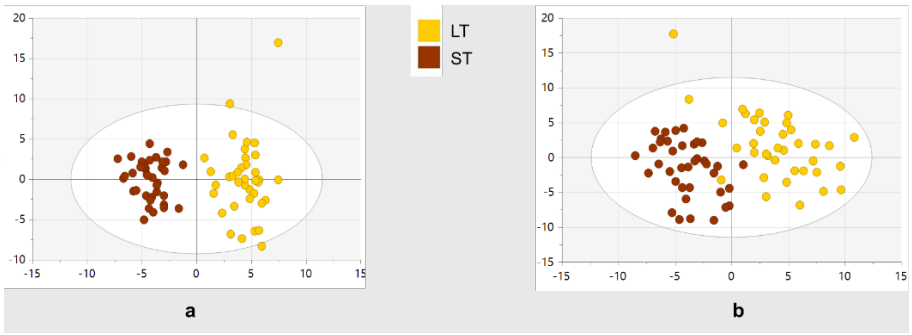

Figure 2. OPLS-DA (a) and feature-reduced PCA (b) score plots of lamb *m. longissimus thoracis* (LT) and *m. semitendinosus* (ST) regardless of the rearing regime.

IV. CONCLUSION

Results showed that rearing system had few major effects on the metabolite fingerprints of dairy lamb meat. Although AR and NR could be differentiated, overall they resulted in similar metabolite composition in meat. Further, REIMS fingerprinting can reliably and quickly discriminate muscle types, and to a lesser extent, different rearing regimes.

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THE USE OF MALDI-TOF MS FOR MICROBIAL IDENTIFICATION OF DISCOLORED VACUUM-PACKAGED BEEF

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I. INTRODUCTION

A significant improvement in tenderness and palatability of beef can be achieved when sub-primal cuts are stored in vacuum-packaging for around 7 and 21 days [1]. But, if red meat muscles attain pH_u values higher than 5.9, this can drive to a superficial green discoloration, probably due to microbial growth [2]. The multiplication and dissemination of bacteria in meat can potentially result in visible color defects [3]. Therefore, bacterial quantification and identification can help to explain probable discoloration problems in meat. For this purpose, MALDI-TOF MS is considered as a fast and reliable identification method, with a high degree of agreement at a genus level with more sophisticated analysis such as 16S rRNA genomic sequencing [4, 5].

II. MATERIALS AND METHODS

Six discolored muscles were identified and collected at 4 days post-mortem from a cattle specialized slaughterhouse located in Niedersachsen, Germany. The muscles were a combination of *M. gluteobiceps, M. tensor fasciae latae*, and *M. lateral vastus*. After breakage of the plastic film, 10g of meat were sterile cut from the part of the muscles where discoloration was notorious and were immediately repackaged in vacuum, kept on refrigeration, and further used for total viable counts (TVC), anaerobic counts and MALDI-TOF (MS) identification. Then, meat color was determined using a ColorLite sph870 colorimeter (Katlenburg Lindau, Germany) set at 45°/0° measuring geometry, 8 mm aperture size, D65 illuminant and 10° aperture, in 15 different points of the muscles. Meat pH was also measured by inserting a Testo 250 pH-meter (Lenzkirch, Germany) in three different points of the muscles. Both pH and meat color were recorded from the average values. The same procedure was repeated for six control samples. statistical analysis of the data was carried out using SPSS software (version 23.0, IBM Corporation, NY, USA).

III. RESULTS AND DISCUSSION

Table 1 – Average quality parameters for discolored (n=6) and control (n=6) meat samples

Parameter	Discolored meat	Control
pH	5.60±0.48[a]	5.66±0.12[a]
L*	36.75±5.11[a]	20.27±4.27[b]
a*	17.42±2.51[a]	26.57±4.03[b]
b*	18.09±5.15[a]	16.54±2.38[a]
TVC (CFU/g)	3.7E+03	5.22E+03
Anaerobic counts (CFU/g)	1.61E+03	2.12+E03

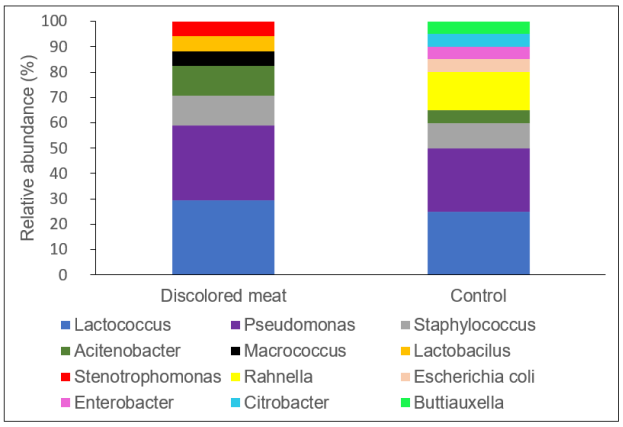

Figure 1: Results of the bacterial identification of discolored (n=6) and normal meat samples (n=6) using the MALDI-TOF (MS) method

There were not significant differences in the pH values of the two groups of meat. The color values showed that *L** was significantly higher, and *a** significantly lower in discolored than in control samples. TVC and anaerobic counts were similar between the two sets of samples. The MALDI-TOF bacterial identification suggested only a minimal difference in the bacterial communities growing in discolored and in control beef.

IV. CONCLUSION

Due to low bacterial counts in the discolored and control meat, it is difficult to assume that the color difference between these two groups of muscles was originated due to microbial contamination. The bacterial identification showed that the microorganisms were bacteria that normally grow under anaerobic conditions in vacuum-packaging. These microorganisms are not commonly related to discoloration processes in fresh bovine muscles. In the future, this project will be extended to a greater number of samples and to a longer time span

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Validation trials for the prediction of water holding capacity of pork meat by vision and VisNIR spectroscopy

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I. INTRODUCTION

There is some focusing interest in the French pork meat sector to test new parameters for carcass grading in slaughterhouses, and water holding capacity evaluation could be the next parameter to be added to carcass weight and lean meat percentage. Many feasibility studies have been conducted at IFIP in the last ten years for the prediction of water holding capacity with vision systems, near infrared spectroscopy and more recently hyperspectral imaging systems. A large validation project is now needed to compare technologies, from accuracy, integration and traceability concerns. The aim of the DATAPORC project is to build robust prediction models involving 7 devices designed for industrial purpose and to validate them on a large population (carcasses, bone-in hams, or deboned hams, depending on the device design). Experiments were conducted in 4 slaughterhouses on a three-year period, with a total of 5562 carcasses. The project also deals with traceability concerns, in order to give consistent feedback to pig producers. This paper presents the results obtained in the first slaughterhouse on deboned hams with two technologies: Visible and Near InfraRed (VisNIR) spectroscopy with contact probe and color evaluation with vision system.

II. MATERIALS AND METHODS

A population of 1251 fresh pork hams were selected during 16 days of trials, according to the *Semimembranosus* ultimate pH (<5.5: n=241 / 5.51-5.7: n=435 / 5.71-5.90: n= 334 / >5.90: n=241). After deboning (24h *p.m.*), the inside of the *Semimembranosus* muscle was measured with a VisNIR spectrometer using a contact probe (ASDI Labspec4, 350-2500 nm). The CSB-Jamboflash vision system was used on the inside of *Semimembranosus* to produce calibrated L*a*b* data, and the built-in calibration was applied to perform the “PSE-like zone” defect grading [1]. The reference meat quality parameters were measured at 24h *postmortem* on the *Semimembranosus* muscle (ultimate pH, color L*a*b*, drip loss (EZ sampling, 48h draining [2])), and the subjective “PSE-like” zone grading was performed on entire deboned hams [3]. The last 6 days of trial were used to build the external validation data set (n=435), whereas the first 10 days were considered as the calibration data set (n=789). Multiple regression models were determined with RStudio (2022.12.0 version) using color data sets from CSB-Jamboflash. PLS and PLSDA models using VisNIR spectra were determined by random cross validation using Matlab 7.10.0 software (Natick, USA) and Eigenvector toolbox (Manson, USA).

III. RESULTS AND DISCUSSION

The meat quality level of samples (pH24=5.71, drip loss=3.9% and PSE-like zone frequency=17.2%) was representative of the standard population [4,5], despite the pH24 sorting process. The overall error of the PSE-like zone grading obtained with the vision system was low (11.5%) with limited false negative ratio (15.1%, table 1) confirming the robustness previously observed (17.1% of false negative, n=9584, [1]). The vision system correlations in external validation for pH24 and drip loss (r=0.65 and 0.66, respectively) were similar to correlations found with the colorimeter L* values (r=-0.67 and 0.64, respectively) showing that vision systems could take advantages of the prediction potential of color for meat quality prediction.

The VisNIR spectrometer PLS prediction models for pH24 and drip loss showed higher calibration R^2 than vision system with very low fitting losses in external validation (R^2_p =0.67 and 0.58 respectively).

The prediction errors were stable (cross validation vs external validation) revealing high precision level for VisNIR spectrometry. The PLSDA model for the PSE-like zone grading with VisNIR showed similar accuracy level (10.2% false negative) than vision system in external validation but was only tested in a single slaughterhouse, unlike the CSB-Jamboflash.

Table 1 – prediction of meat quality of pork ham with VisNIRS (ASDI Labspec4) and Vision (CSB Jamboflash) applied on *Semimembranosus* muscle

	Labspec4 (ASDI)							CSB-Jamboflash			
	calibration (n=794)					External validation (n=436)		Linear regression (n=789)		Prediction (n=435)	
	Spectral treatment*	Nb PLS factor	R^2_c	R^2_{cv}	$rmse_{cv}$	R^2_p	$rmse_p$	R^2	rmse	R^2_p	$rmse_p$
pH24	GLS weighting	2	0.73	0.65	0.13	0.67	0.14	0.55	0.15	0.42	0.17
Drip loss (%)	SNV	5	0.55	0.54	1.8	0.58	1.8	0.42	2.04	0.44	2.06
	Spectral treatment*	Nb PLSda factor	R^2_c	R^2_{cv}	$rmse_{cv}$	False positive (%)	False negative (%)	Prediction (n=1251)			
								False positive (%)		False negative (%)	
PSE-like zone (%)	1st derivative	1	0.44	0.41	0.42	8.9	10.2	10.7		15.1	

*: *smoothing+baseline correction+treatment*

IV. CONCLUSION

This first data treatment extract from the DATAPORC project showed that proper calibrations of water holding capacity prediction with vision system and VisNIR spectrometry give enough accuracy in external validation for carcass batch grading. The VisNIR spectrometer accuracy is higher than vision system, but it needs meat contact and an operator. The deboned status of the meat here is clearly an advantage for the prediction but the traceability is difficult to maintain. The next DATAPORC data sets will compare these technologies on bone-in meat cuts or carcasses, with other muscle measurement opportunities but with easier traceability control.

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INFLUENCE OF CARCASS VASCULAR RINSING WITH CALCIUM CHLORIDE ON NON-ELECTRICALLY STIMULATED AND STIMULATED CARCASSES ON MEAT QUALITY OF LAMBS

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I. INTRODUCTION

Infusing calcium chloride into carcasses before rigor mortis presents a promising avenue for enhancing meat quality and tenderness. This technique targets the activation of μ-calpain and m-calpain, two key enzymes responsible for the postmortem breakdown of myofibrillar and cytoskeletal proteins (Huff Lonergan et al., 2010). The effectiveness of intra-arterial infusion in non-stimulated carcasses with a 0.3 M solution of $CaCl_2$ (10% live weight) was previously demonstrated (beta-adrenergic agonist fed lambs), resulting in reduced shear force across varying postmortem intervals (1, 7, 14 days; Koohmaraie et al., 1991). The emergence of Rinse & Chill® technology (MPSC Inc., Hudson, United States) has further facilitated the commercial viability of calcium chloride infusion into carcasses, fostering opportunities for continued exploration and application of such methods (Hwang et al., 2022). This study aimed to assess the impact of calcium chloride infusion into lamb carcasses on pH decline, meat color, and Warner-Bratzler shear force (WBS). It was hypothesized that the infusion of calcium chloride coupled with electrical stimulation would lead to a reduction in shear force, thereby contributing to increased meat tenderness.

II. MATERIALS AND METHODS

The study was conducted over three different trial periods with lambs (n=40) which consisted of various breeds (commercial crossbred, Dorset, Hampshire, Suffolk, Polypay, Southdown), age (6-9 months), and live body weight (59.4 ±29.5 kg). Lambs were randomly assigned to three vascular rinse treatments (TRT) that included: (RC= Rinse & Chill® solution; saccharides, phosphates), (CA= 0.3M $CaCl_2$ + RC), and (ES-CA= electrical stimulation, 800mA current, peak 350V for 60 seconds, followed by CA). Animals were stunned by penetrating captive bolt. The vascular rinsing process entailed inserting a catheter into the heart and rinsing the carcass at 10% of its body weight. Treatments were applied to the carcass immediately upon exsanguination. Carcasses were skinned, eviscerated, and chilled (3℃, 24 h). Carcass temperature and pH were recorded (semimembranosus, SM) from 1 to 20 h postmortem (PM). At 24 h PM, the longissimus dorsi (LD), SM, and triceps brachii (TB) were excised, vacuum packaged, or overwrapped in oxygen-permeable film. Color measurements (CIE L*, CIE a*, chemical states of myoglobin) were determined during storage (3 and 7 d PM). Purge, Warner-Bratzler shear force (WBS) on cooked (68.3 ℃ internal) LD, SM, and TB chops, and cook loss (3 and 7 d PM) were determined. Data was analyzed as a split-split plot design with means ($P<0.05$) separated using PROC MIXED (SAS Institute).

III. RESULTS AND DISCUSSION

Live animal weights and hot carcass weights were not different ($P>0.05$) among the treatments. For carcass pH, ES-CA treatment was lower (5.59, $P<0.05$) than the CA (5.69) and RC (5.85) treatments, while CA was lower ($P<0.05$) than RC (Figure 1). The lower pH, indicative of more rapid and extensive glycolysis observed in ES-CA can be attributed to the muscles repeatedly contracting and relaxing because of electrical stimulation along with the elevated calcium level in the sarcoplasm further enhancing contraction of the muscles. RC had higher ($P<0.05$) CIE L* values than CA and ES-CA by 1.9 and 2.3 units, respectively. Additionally, RC showed greater ($P<0.05$) CIE a* values compared to CA and ES-CA by 1.5 and 1.4 units, respectively, and higher Chroma C values by 2.3 and 2.2 units, respectively. Furthermore, RC exhibited greater ($P<0.05$) oxymyoglobin levels than CA and ES-CA by 3.6% and 2.5%, respectively, while metmyoglobin was lower ($P<0.05$) in RC compared to CA and ES-CA by 4.5% and 4.8%, respectively.

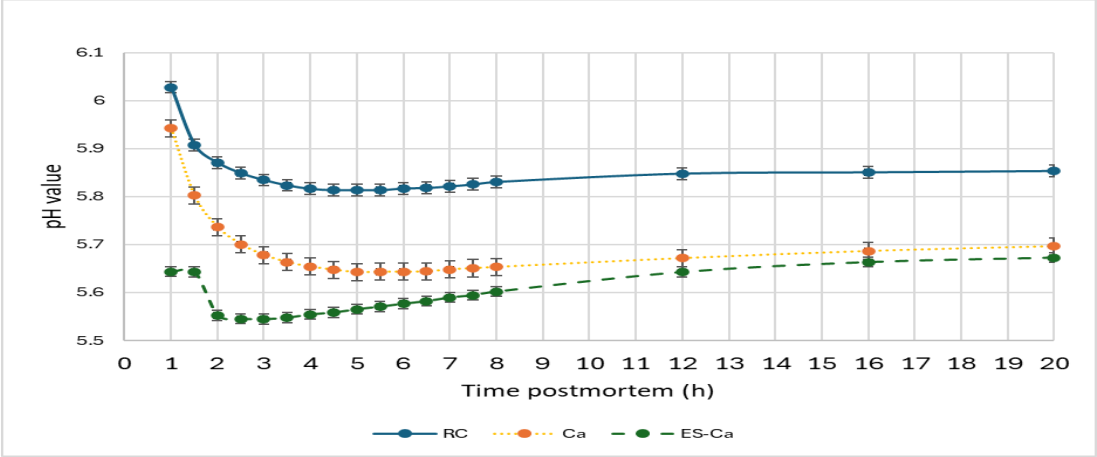

Figure 1. Carcass pH of each treatment group at hours 1-20 postmortem (PM)

The SM muscle had lower CIE L* (P<0.05) than the LD and TB muscles. At day 3 PM, CIE a* was lower (P<0.05) in the LD muscle compared to TB and SM. On day 7 PM, CIE a* was higher (P<0.05) in the TB muscle compared to LD and SM. TB from the RC had the least purge in the LD and SM compared to CA and ES-CA. RC had lower (P<0.05) cook loss than ES-CA by 3.0%. In CA and ES-CA, the LD showed lower (P<0.05) WBS values than RC by differences of 28.2% and 34.8%, respectively. With ES-CA, the SM exhibited the lowest (P<0.05) WBS. RC had the greatest WBS at day 3 (P<0.05), with no differences (P>0.05) found between TRT on day 7 (Table 1).

Table 1. Least square means of cooked lamb chops from vascularly rinsed carcasses on Warner-Bratzler Shear (newtons)

	Muscle			Day	
TRT	LD	SM	TB	3	7
RC	29.64^{b}	36.49^{a}	27.36^{b}	35.66^{a}	26.67^{b}
CA	22.31^{c}	34.60^{a}	29.73^{b}	28.77^{b}	28.99^{b}
ES-CA	20.85^{c}	30.14^{b}	29.26^{b}	26.69^{b}	26.81^{b}

$^{a-c}$Means with unlike letters within treatment and muscles are different (P<0.05, TRT*Muscle interaction, S.E.= 1.452)
$^{a-b}$Means within treatment and day with unlike letters are different (P<0.05, TRT*Day interaction, S.E.= 1.229)

IV. CONCLUSION

The implications of vascularly delivering calcium chloride throughout the carcass on meat quality are noteworthy, particularly in shear force reduction, suggesting the potential to enhance tenderness. More rapid chilling to reduce the effect of the rapid drop in pH may benefit meat color and moisture retention.

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HYPERSPECTRAL IMAGING CAN BE A RAPID TOOL TO MONITOR WEIGHT LOSS AND QUALITY CHANGES DURING DRY-AGING OF BEEF

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I. INTRODUCTION

Dry-aging is a processing technique used to enhance the flavor and eating quality of meat. This process involves various changes such as weight loss, oxidation, and microbial activities, which play a significant role in shaping the flavor of dry-aged meat [1]. Understanding the evolution of these parameters during the dry-aging process and developing a non-invasive method to accurately predict meat quality and flavor intensity is essential to optimize processing strategies. This will help to produce dry-aged meat with consistent and guaranteed quality, resulting in a more satisfying eating experience. This study aimed to apply hyperspectral imaging (HSI) to monitor the weight loss, moisture content, pH, instrumental color and lipid oxidation level during dry-aging of beef for up to 21 days.

II. MATERIALS AND METHODS

Twenty-four striploins from dairy-beef crossbred yearling calves (~12 months, n=12) and 2-year-old cattle were obtained at 48 h post-mortem, cut into four sections, and assigned to in-bag dry-aging (BD) for 0, 7, 14, and 21 days at 2°C and 75% relative humidity. The weight losses during BD were recorded. At each time point, steaks were taken from each section and determined for pH and instrumental color (Lightness, redness, chroma, and hue angle) according to the previous study by Zhang et al [2]. The same steaks were then analyzed by HSI as described below. The moisture content (freeze-drying) and thiobarbituric acid reactive substances (TBARS) values of these steaks were also determined [2]. A linescan HSI system was used in this study which included a translation stage, an illumination system, and a hyperspectral camera [3]. The hybrid camera sampled 235 wavebands with a spectral range of 550nm to 1700nm and a spectral resolution of 5 nm. A linear translation speed of 11.1mm/s was chosen to ensure square pixels in the image. The stand-off distance between the sample and the lens was set to ~ 370mm. Each hyperspectral image was calibrated with white and dark reference measurements for obtaining the reflectance values. One hyperspectral image was acquired for each sample on day 0, 7, 14, and 21. A total of 96 images (24 samples × 4 ageing times) were captured. Raw hyperspectral images were subjected to data pre-processing and multivariate analysis using R and Python. Hyperspectral images were cleaned to remove background pixels and averaged into a single spectrum per sample. The obtained raw spectral data was pre-processed using standard normal variate (SNV) transformation followed by Savitzky-Golay (SG) smoothing (window size=5) to remove any baseline effects and enhance signal-to-noise ratio (SNR) [4]. The pre-processed spectral data along with the reference data was subjected to PLSR analysis to develop and validate prediction models for individual attributes, using the repeated double cross-validation (rdCV) method [5].

III. RESULTS AND DISCUSSION

Table 1 illustrates the results of PLSR models for various dry-aged beef quality attributes using rdcv strategy. Weight loss model showed the best performance, yielding a R^2 of 0.92 and SEP of 0.03% followed by moisture content, redness, chroma, yellowness and hue angle models. pH and TBARS values showed poor results which could be related to less variability in pH across samples and absence of spectral peaks associated with TBARS. Selectivity ratio (SR) plots showed that weight loss model was governed by 3 major peaks: 789nm which could be related OH 3rd overtone or an

absorption band produced by deoxymyoglobin, 1036nm and 1390nm which could be due to OH 2nd overtone related to water. Moisture model had a major peak around 1376nm related OH 2nd overtone [6]. All color related models including redness, chroma, yellowness and hue angle showed major peaks around 818nm related to OH 3rd overtone or deoxymyoglobin, 1400nm related to water and 1540nm which is highly overlapped and influenced by water (OH) and protein (N-H and C-N stretching) bands [7].

Table 1. PLSR model results using repeated double cross-validation strategy.

Attributes	n	R^2	No. of PLS Components	SEP
Weight loss	96	0.92	4	0.03
Moisture (freeze-dry)	96	0.78	3	1.55
Redness	96	0.75	3	1.80
Chroma	96	0.72	2	2.09
Yellowness	96	0.61	2	1.13
Hue angle	96	0.59	3	1.78
pH	96	0.34	6	0.07
TBARS	96	0.33	2	0.08

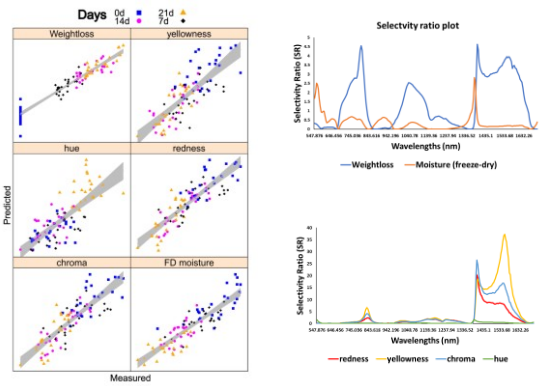

Figure 1. Prediction plots (left) and Selectivity Ratio (SR) plots (right) for quality attributes of dry-aged beef for 0, 7, 14, and 21 days.

IV. CONCLUSION

The results showed that HSI can be used to predict dry-aged beef's quality attributes including weight loss, moisture content, and color throughout the aging time up to 21 days. Further exploration revealed that models were majorly utilizing chemical information related to water in weight loss and moisture models whereas color parameters were predicted by a combination of color pigment (myoglobin) and water and/or protein. HSI was able to overcome the heterogeneity of meat samples and could be a potential tool for real-time, rapid, and non-invasive assessment of dry-aged beef quality for consistently high-quality product.

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A dietary sugarcane-derived polyphenol mix reduces enteric methane emissions and improves meat quality in pasture-fed beef cattle

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I. INTRODUCTION

Strategies to use feed additives to reduce methane emissions and improve meat quality is a novel area of research as part of responsible meat production. Previous studies have demonstrated that plant-based polyphenols can reduce enteric methane emissions [1] and possess antioxidant capability to improve the shelf life of meat [2]. Recent research from our lab has demonstrated the anti-methanogenic potency of polyphenols in sheep [1], and heat stress mitigation with improved meat quality in chicken.[3]. The present study investigated the effects of sugarcane-derived polyphenol supplementation (SDP) on the enteric methane emissions and meat quality of pasture-fed beef cattle.

II. MATERIALS AND METHODS

The animal study was approved by the University of Melbourne Animal Ethics Committee. Twenty-four Angus steers (avg age 6 months) were selected for 6 months study and randomly allocated to two groups (N=12) CON: Lucerne pellets, SDP: commercial sugarcane-derived polyphenol supplement incorporated into Lucerne pellets (@ 0.25%/Dry matter). Pellets were delivered using automatic feeders (Lely® Netherlands) placed in each paddock while animals were grazing perennial pastures. Enteric methane emissions were measured via Guardian NG gas cards installed on the automatic feeder. Body weights were recorded every month over 6-month study period starting with weaners (approx. 220±20 kg). At the end of the grazing and supplementation study, steers were slaughtered at a commercial abattoir, and carcass quality and MSA data were recorded. Ultimate pH was measured and M. longissimus lumborum (LL) and M. psoas major (PM) from the left side of the carcasses were collected 24 hrs postmortem. For retail color stability, each muscle was cut into pieces with an average weight of 120±10 grams packed in aerobic packaging and placed in a 4-6 ºC refrigerator cabinet with LED internal lighting to simulate the retail display conditions. Meat color (L*, a*, b*) was measured each day using a Minolta colorimeter. Drip loss was measured at day 0 by the EZ-drip loss method, purge loss was measured between days 0 and 1,3,5, and 7. Cooking loss and Warner-Bratzler Shear Force (WBSF) were measured on days 0,1,3,5 & 7 using a texture analyzer. Lipid oxidation was assessed for all days by TBARS procedure as described by Sørensen and Jørgensen [4]. Statistical analysis was performed using Genstat (22nd edition) by the method of restricted maximum likelihood (REML) based on multiple factors (pellets fed, muscles, display days). Multiple comparisons were conducted using Tukey's test.

III. RESULTS AND DISCUSSION

The effect of feeding of SDP was highly significant (p<0.001) in reducing total methane production without any adverse impact on growth rate and carcass characteristics. There was a significant (p<0.001) effect of dietary treatment on meat lipid oxidation and lower TBARS values were observed in the meat of animals fed lucerne pellets with SDP as compared to the CON group (Table 1). In CON, the malonaldehyde (MDA) value in LL was lower on day 1 and then increased between days 3, 5, and 7. Dietary treatment had a significant effect on cooking loss, and meat of animals fed lucerne pellets with

SDP showed lower cooking loss. Purge loss was also significant, and the SDP group had lower purge loss throughout the display for both LL and PM. Warner-Bratzler Shear Force values were higher for LL in the SDP treatment group while lower for PM in comparison with CON and interaction between day and treatment was significant (p<0.05).

Table 1- Mean (SED & P Values) Lipid oxidation, WBSF, Cooking Loss, and Purge Loss of beef muscles during retail display.

			Days						p-Values						
Parameter	T	M	0	1	3	5	7	SED	Days	M	T	D*M	D*T	M*T	D*M*T
Lipid Oxidation (MDA/kg)	CON	LL	0.178	0.210	0.562	0.622	0.884	0.060	<0.001	0.038	<0.001	0.001	<0.001	0.905	0.775
		PM	0.140	0.284	0.531	0.677	1.032								
	SDP	LL	0.237	0.360	0.493	0.592	0.441								
		PM	0.147	0.515	0.477	0.589	0.579								
WBSF (N)	CON	LL	30.57	26.5	19.4	21.18	16.43	1.374	<0.001	<0.001	0.22	<0.001	0.043	0.002	0.171
		PM	24.03	22.3	21.1	21.86	18.55								
	SDP	LL	33.59	31.22	21.52	18.56	20.38								
		PM	23.09	21.88	19.99	20.56	20.02								
Cooking Loss (%)	CON	LL	21.56	28.21	20.85	26.11	22.43	0.917	<0.001	0.527	<0.001	0.009	0.086	0.323	0.092
		PM	22.19	27.36	22.99	24.63	21.34								
	SDP	LL	18.25	25.71	20.16	24.01	21.8								
		PM	21.38	25.28	19.87	23.56	22.06								
Purge Loss (%)	CON	LL	------	1.248	0.698	1.188	1.789	0.2815	<0.001	0.351	0.007	0.432	0.014	0.129	0.631
		PM	------	1.332	0.694	1.868	2.062								
	SDP	LL	------	0.707	0.627	1.738	1.368								
		PM	------	0.493	0.513	1.684	1.568								

SED: Standard Error of Difference
T: Treatment
M: Muscle
D*M: Day x Muscle
D*T: Day x Treatment
M*T: Muscle X Treatment
D*M*T: Day x Muscle x Treatment

IV. CONCLUSION

This study has shown that dietary sugarcane-derived polyphenol supplementation reduces enteric methane emissions and improves the meat quality characteristics such as lower cooking loss, purge loss, and lipid oxidation while enhancing retail shelf life. Consequently, it can be suggested that sugarcane-derived polyphenol is a suitable feed additive for mitigating GHG emissions and enhancing meat quality in grazing beef cattle.

ACKNOWLEDGEMENTS

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TOWARD OBJECTIVE MEAT QUALITY EVALUATION: MULTISPECTRAL MACHINE VISION APPROACH

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INTRODUCTION

Meat color and intramuscular fat (IMF) evaluation can involve subjective and objective assessment methods. Subjective assessment often employs the use of the National Pork Producers Council (NPPC) color standards, or similar subjective measurement methods. Objective measurement with devices like the Minolta Chroma Meter provides a single value but doesn't distinguish between meat and fat accurately. The primary objective of this work was to investigate the possibility of generating a continuous score for quantifying whole–slice meat quality (color and marbling) using hyperspectral imaging, aiming for high quality data for a pig breeding program.

MATERIALS AND METHODS

Pork *longissimus dorsi* muscle (fresh [n=22] and frozen [n=41]) and fresh ham portions (*gluteus medius* [n=20] and *gluteus profundus* [n=20]) were collected. The hyperspectral camera employed for capturing Hyperspectral Images (HSI) is the Specim IQ, facilitated by Specim's push-broom technology, and a standard imaging resolution (512x512 pixels). Two halogen light sources (D65) were used from a 45-degree angle, and the camera was placed perpendicular to the sample at a height of approximately 20 cm. A reference target with a known reflectance was placed for calculating the incident light intensities, which was later used for calculating the reflectance. Using these settings, HSI were captured on all samples, with each HSI dataset having 204 bands with a spectral resolution of 2.9 nm, and a wavelength range extending from 400 nm to 1000 nm. Each slice was also evaluated for whole number NPPC color and marbling values, determined by a trained expert, and a corresponding decimal NPPC value ($NPPC_d$). These parameters served as the foundational elements for the comprehensive evaluation of pork meat quality in this study. The data distribution is illustrated in Figure 1 below.

Data analysis

The objective is to extract a meat-only spectral signature for each meat slice. As observed, the meat slice is composed of roughly three spatial regions: meat, subcutaneous fat, and IMF. Observations of the spectral signatures of these three regions, within the same slice and throughout different slices, confirm that a different spectral signature exists between these three regions. More specifically, variability in the level of the spectral signature, but not of the signature itself, was observed (Figure 1).

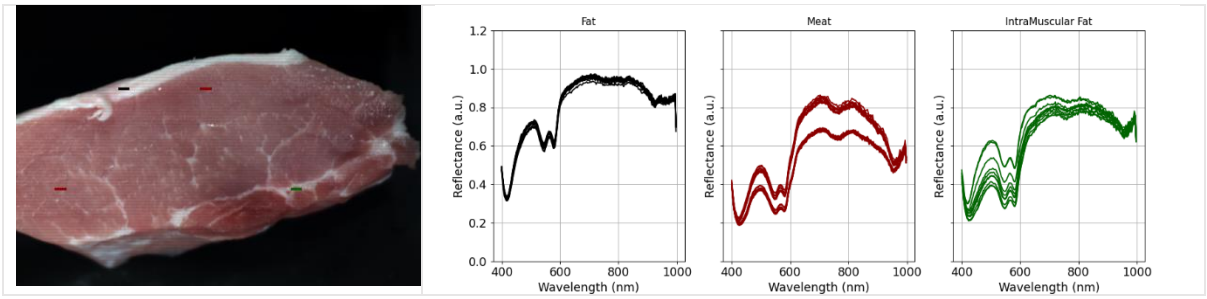

Figure 1. Spectral signatures (right) of manually delineated regions within one pork slices (loin, left, RGB representation). Three regions covering subcutaneous fat (black), meat (red) and intramuscular fat (green) were extracted.

Based on this observation, a fat extraction method was implemented based on the hypothesis of spectral invariance within each region. Two spectral regions, defined by lower and higher wavelength boundaries, were defined as the “low wavelength band” and “high wavelength band”. For each pixel of

the meat slice, the spectral response was averaged within these two regions. The ratio between these two spectral regions was then computed for each pixel: closer to identity for pixels with a “fat” spectral signature, and higher for pixels with a “meat” spectral signature. Finally, a simple threshold, empirically set to 2, allowed to generate pixel masks for the fat regions (<=2) and meat regions (>2).

As our objective is to quantify a subjective visual perception of meat quality, the analysis was constrained to wavelengths in visible spectra, from 400nm to 850nm, ended up in 154 wavelengths.

Statistics

A spectral signature was generated for the 103 pig meat slices (Figure 2). Based on this spectral signature, a simple linear regression model was fitted where the explanatory variables were the 154 bands and the NPPC or $NPPC_d$ were the variables to explain.

As the wavelength bands are quite narrow, there is high correlation between variables. In this work, the dimension was reduced from 154 variables to 3 variables. To find these variables a brute force technique was used: a linear model was fitted to each 3-wavelength combination, and the combination giving the highest goodness-of-fit was considered the optimal choice of bands. A Leave One Out (LOO) validation scheme was implemented to measure the quality of the model. In such scheme, one model is fitted using all but one slice in the dataset. The model obtained is then used to predict the slice that was not used in the fitting step. Once done for all data points, the metrics such as goodness-of-fit (R^2) and mean absolute percentage error (MAPE) were calculated between the predictions and the variables to explain (NPPC or $NPPC_d$).

RESULTS AND DISCUSSION

The 3 bands found to give the best results are 576.5nm, 723.5nm and 782.4nm. Figure 2 (middle) shows the R^2 obtained with all the 3-wavelength combinations, in the order tested and sorted from best to worse.

Using these three bands, the predicted $NPPC_d$ are plotted against the true $NPPC_d$ in Figure 2 (right). The group information was added to the drawing to show that there is no difference in prediction between the groups. The correlation between predicted and true is R^2=0.87. This gives confidence that the spectral signatures contain enough information to represent the meat quality as scored by meat quality experts.

Hyperspectral cameras enable imaging of narrow spectral bands across a continuous spectrum range, producing spectra for all pixels in the scene. This novel method provides a machine vision-based assessment of meat color that reflects meat quality as perceived by consumers.

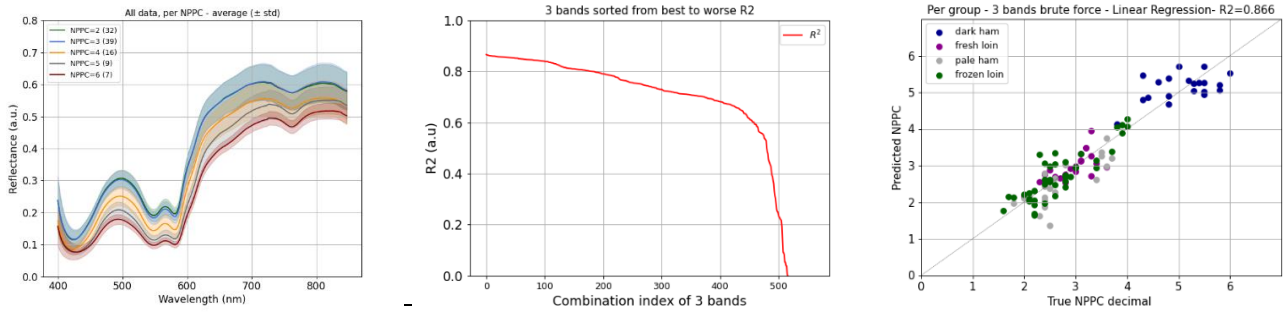

Figure 2. Left: All 103 spectral signatures per NPPC, represented with the mean and std of each group. Middle: Validation metrics (R^2) obtained with all the combinations of 3 bands, ordered from best to worse. Right: Predicted NPPC (y-axis) plotted against the true $NPPC_d$ (x-axis), optimal three bands.

CONCLUSION

The project has demonstrated the feasibility of developing a method for evaluating meat quality using non-invasive multispectral machine vision, based on meat color and marbling measured with a hyperspectral camera. The proposed method demonstrates excellent segmentation capability and provides an objective measure of fat and meat color akin to human visual perception.

PREDICTING BEEF CARCASS COMPOSITION USING DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) SCANNING OF DIFFERENT CARCASS SECTIONS

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I. INTRODUCTION

The quantification of body composition is important to estimate animal nutritional requirements and saleable meat yield [1]. Dissection is a direct method to quantify tissue composition. However, it is not feasible to perform in an industrial environment due to its laborious and time-consuming nature. As a reliable indirect method, DXA scanning has been used to accurately predict lean, fat, and bone tissues [2,3,4]. We hypothesized that carcass physical composition can be precisely estimated using DXA data of different carcass sections. Therefore, this study aimed to develop equations to predict the half carcass composition of young Nellore males using DXA scanning of different carcass sections.

II. MATERIALS AND METHODS

The right half-carcasses from 18 young Nellore males (9 bulls and 9 steers), receiving maintenance (n = 6), high (n = 6), or low (n = 6) concentrate diets were evaluated in this study. The treatments were selected to maximize the range in hot carcass weight and fat score. After 24 h of chilling, carcasses were divided into five sections (Figure 1), and then scanned in a medical DXA unit (GE Healthcare, Lunar Prodigy Advance, USA). The Small Animal configuration mode of the GE Healthcare enCORE software, version 18, was selected. The DXA scanning provided data on fat, lean, and bone mineral content (BMC). Afterward, each section was dissected into fat, muscle, and bone content. DXA variables (fat, lean, and BMC) of carcass sections were used to predict carcass physical composition (fat, lean, and bone) using general linear regression models in SAS (Institute Inc., Cary, NC, USA). To develop prediction equations based on the input variables, a leave-one-out cross-validation method was performed. The precision of the predictions was assessed based on the coefficient of determination (R^2) and root mean square error (RMSE).

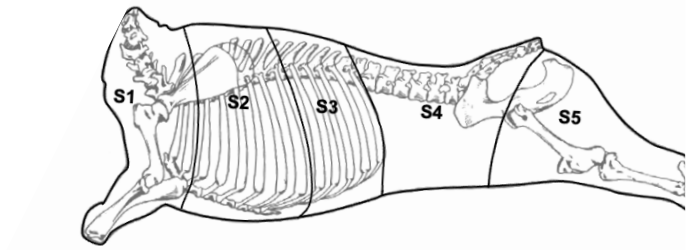

Figure 1. Representation of carcass sections used to predict half carcass composition. S1 - longitudinal cut after the second rib, S2 - longitudinal cut after the eighth rib, S3 - longitudinal cut after the thirteenth rib S4 - longitudinal cut after the sacrum, and S5 - the remaining section after the removal of S1 to S4

III. RESULTS AND DISCUSSION

The predictive equations for carcass physical composition, based on DXA measurements of different carcass sections, are shown in Table 1. Prediction of carcass muscle and bone using DXA data from section 1 exhibited lower RMSE and AICc values, coupled with high R^2 values, when compared to the equations based on the other evaluated sections. However, the equation for carcass fat prediction, using DXA fat content of section 5, showed the lowest RMSE and AICc values among the evaluated sections.

Table 1 – Prediction equations for carcass physical composition using dual energy X-ray absorptiometry (DXA) of carcass sections.

Tissue	Regression equation	R^2	RMSE	AICc
Muscle (kg)	Carcass muscle = 0.925 + 0.808 x total DXA lean[1]	0.92	5.56	85.36
	Carcass muscle = 5.275 + 2.386 x DXA lean S1	0.93	5.22	83.11
	Carcass muscle = 11.218 + 4.873 x DXA lean S2	0.70	11.05	110.08
	Carcass muscle = 11.069 + 7.658 x DXA lean S3	0.88	6.89	93.08
	Carcass muscle = 8.928 + 5.352 x DXA lean S4	0.79	9.18	103.42
	Carcass muscle = 2.878 + 2.424 x DXA lean S5	0.84	8.12	98.99
Fat (kg)	Carcass fat = 1.069 + 0.864 x total DXA fat[1]	0.96	1.89	46.58
	Carcass fat = 4.961 + 2.795 x DXA fat S1	0.83	4.18	75.09
	Carcass fat = 6.537 + 3.561 x DXA fat S2	0.75	4.99	81.53
	Carcass fat = 4.690 + 5.516 x DXA fat S3	0.88	3.56	69.28
	Carcass fat = 3.475 + 4.095 x DXA fat S4	0.82	4.24	75.64
	Carcass fat = -0.497 + 3.703 x DXA fat S5	0.92	2.86	61.41
Bone (kg)	Carcass bone = 6.446 + 2.478 x total DXA BMC[1]	0.88	1.33	33.83
	Carcass bone = 6.066 + 7.646 x DXA BMC S1	0.90	1.23	31.26
	Carcass bone = 8.233 + 15.583 x DXA BMC S2	0.71	2.09	50.19
	Carcass bone = 9.155 + 22.079 x DXA BMC S3	0.68	2.16	51.24
	Carcass bone = 11.295 + 12.732 x DXA BMC S4	0.67	2.24	52.63
	Carcass bone = 6.111 + 7.523 x DXA BMC S5	0.82	1.66	41.81

[1]Equation developed using pooled data from all carcass sections. S1 = section 1; S2 = section 2; S3 = section 3; S4 = section 4; BMC = bone mineral content; RMSE = root mean square error; AICc = corrected Akaike's information criterion.

Evaluating the developmental pattern of beef steers during fattening, Luitingh [5] demonstrated that shoulder and round represent the earliest-maturing parts of the carcass. This development pattern may explain the reason why sections 1 and 5 provided better estimates of carcass physical composition.

IV. CONCLUSION

Section 1 equation for muscle and bone and section 5 equation for fat content have shown the most precise estimates of half carcass tissue composition. However, more studies are necessary since our equations are based on Nellore bulls and steers and low sample size.

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COOKING EFFECT ON ANTIOXIDANT ACTIVITY OF POULTRY MEAT ENRICHED WITH n-3 BY CHIA SEEDS IN THE DIET

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I. INTRODUCTION

Poultry meat is highly consumed worldwide and is a good source of proteins, essential amino acids, vitamins, and fat. Besides, it has more unsaturated fatty acids than red meat [1]. Several strategies have been tested to improve the meat´s nutritional profile to address major public health concerns [2], such as obesity, cardiovascular disease, diabetes, and some types of cancer. Chia seed is a promising source of *n*-3 polyunsaturated fatty acids (PUFA), especially α-linolenic acid, and antioxidant compounds such as flavonoids, anthocyanins, vitamins, and carotenoids [3]. Previous research showed that the inclusion of chia in the diet of broilers and rabbits was able to increase PUFA concentrations and decrease saturated fatty acid (SFA) content in meat [4,5]. During heat treatment application, a high percentage of water is lost, and chemical complexes are developed that alter the chemical structure and favor the oxidation of lipids in the meat. There is not much information about what happens with the antioxidant activity of meat during cooking, therefore, this study aimed to investigate the cooking effect on cooking loss and the antioxidant activity of poultry meat enriched with n-3.

II. MATERIALS AND METHODS

Two hundred one-day-old male Ross chickens were fed a starter diet, corn-soybean base (21.7% CP, 2998 kcal/kg ME). After 21 days, 96 birds were randomly selected and divided into four groups receiving one of the following diets *ad libitum* (iso-proteic and isoenergetic): a corn-soybean base diet (control group), and three groups with the inclusion of 2.5%, 5%, and 10% chia seed. On day 49, all the chickens were sacrificed in a commercial slaughterhouse according to CHEA (Honorary Animal Experimentation Commission, protocol N°702). After chilling, carcasses were transported to the laboratory, and twelve *Pectoralis major* muscles from each group were removed and conditioned in polyfoam trays overwrapped with oxygen-permeable PVC film. Trays were kept in a commercial case (CE, SS1500 model, 1.25 m height, 90 cm wide, 1.50 m long) with artificial light at 2–8°C for 4 days, simulating retail display conditions. Afterward, they were vacuum packaged and frozen at −80°C. Meat samples were cooked by placing 30 g in a vacuum-sealed sous-vide bag in a water tank for 60 minutes at 75°C, chilled in an ice bath for 30 minutes, and then kept at -80°C. The percentage of cooking loss was calculated by the difference in weight of meat samples before and after cooking. Antioxidant activity in raw and cooked poultry meat was determined by the ABTS radical scavenging activity assay [6]. Briefly, 1.5 g of meat was homogenized with 7.5 ml of methanol in an Ultra-turrax (IKA T18 Basic) at 8000 rpm for 20 seconds, and later centrifuged at 4000 *g* for 10 minutes at 4°C. The supernatant was filtered (Whatman N°1) and incubated with the working solution (ABTS) in a stirrer plate (Unimax 1010, Heidolph) at 160 rpm in the dark for 30 minutes. A calibration curve with Trolox was done, and the absorbance was measured in a spectrophotometer (T70 UV/Vis, PG Instruments) at 734 nm. The antioxidant activity was expressed as µmol Trolox Equivalent (TEq)/g of fresh meat. The data were analyzed with the NCSS program. A significance level of $P<0.05$ was established. A one-way ANOVA followed by the Tukey & Kramer test was used to analyze the diet treatment effect for the cooking water loss data and the antioxidant activity data within the raw and cooked meat. A repeated measures ANOVA followed by the Tukey & Kramer test was used to evaluate the main effects of cooking and diet treatment on the antioxidant activity data.

III. RESULTS AND DISCUSSION

The mean values for cooking loss (Figure 1) ranged from 28.91% ± 1.09 in meat from the control group to 31.20% ± 0.95 from the 2.5% inclusion of chia seed group. No differences between poultry meat from the four diet treatments were observed in the cooking loss results.

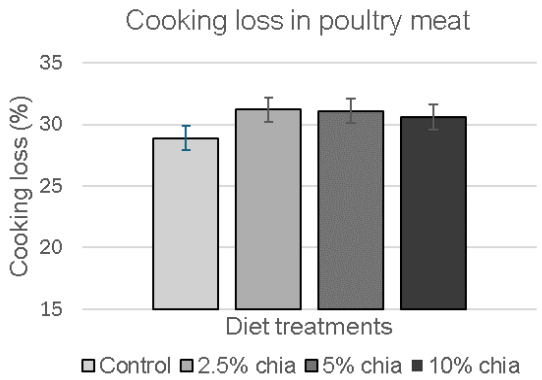

Figure 1. Cooking loss (%) of poultry meat enriched with n-3 by increasing levels of chia seed in the diet (2.5%, 5%, and 10%). Results are mean +/- SEM (n=12).

Antioxidant activity results in raw and cooked poultry meat enriched with n-3 are shown in Figure 2. Cooked poultry meat presented lower values of ABTS than raw meat (main effect, P<0.0001). Within raw meat, no differences between diet treatment groups were found. Despite this, meat from the control group (corn-soy diet) presented a lower antioxidant activity compared to the 10% chia seed group within cooked meat. This result could be explained by the antioxidants present in chia seeds, which could have protected poultry meat from oxidation during the cooking process.

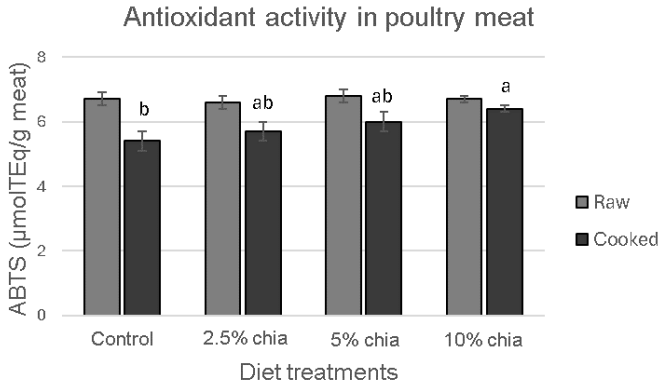

Figure 2. Antioxidant activity measured as ABTS (µmol TEq/g meat) in poultry meat enriched with n-3 by increasing levels of chia seed in the diet (2.5%, 5%, and 10%). Results are mean +/- SEM (n=12). Main effects: diet treatment NS; cooking (P<0.0001, raw>cooked); diet x cooking NS.

IV. CONCLUSION

A 10% chia seed inclusion in the poultry diet can increase the antioxidant activity of cooked meat compared to a corn-soy conventional diet.

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DIGITAL SLAUGHTERHOUSE
Training the next generation of the meat industry

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I. INTRODUCTION

Understanding the role of the official veterinarian in the slaughterhouse and general slaughterhouse operation is a required day-one competency for veterinarians in the UK. However, universities worldwide, including those in the UK, face significant challenges in delivering this essential Veterinary Public Health teaching. These challenges are exacerbated by factors such as a lack of suitable slaughterhouse facilities, food business operators' reluctance to admit students due to biosecurity and perceived commercial risk, and health and safety concerns for students visiting industrial and mechanized environments. Even when access is granted, the teaching content is often inconsistent between groups as it relies on the production line's effective operation on a specific day. Students who have not visited a slaughterhouse can be concerned and anxious about their first visit, resulting in more difficulties in achieving the required learning outcomes as each takes time to acclimate to the environment. A unique academic / industry partnership was established to tackle these challenges, bringing together the University of Glasgow, the Royal Veterinary College, the University of Surrey, and a technology development partner, Denova. This consortium and five meat industry collaborators aimed to design and build a technical resource that complimented live slaughterhouse visits. The objectives were to first prepare students for their visit, reduce concerns and anxieties where possible, and secondly, support veterinary public health students in achieving the Royal College of Veterinary Surgeons (RCVS).

II. MATERIALS AND METHODS

Images, video, and audio from five slaughterhouse facilities, including bovine, porcine, poultry, ovine, and deer, were captured. This multimedia was combined with teaching content carefully curated and provided by six experts in veterinary public health. The teaching content, including streamed videos, was delivered as a browser-based eLearning WebApp, with the 360-degree videos being presented in Virtual Reality(VR), giving the students an immersive introduction to the slaughterhouse environment. The video in the slaughterhouses was filmed during its routine operations with a 360-degree stereo Vuze+ camera. The camera allowed the simultaneous capture of 4K video with both left and right eye views in a full 360 circle and surrounding audio through its 8 lenses and 4 microphones, respectively. When video from this camera is replayed on a VR headset, it allows the user to look in all directions with both left and right eye views, giving the user 3D views of the slaughterhouse. The Digital Slaughterhouse (DS) was built to be a Sharable Content Object Reference Model (SCORM) compatible with Learning Management Systems (LMS), enabling hosting either by individual universities or as a subscription service on a centrally hosted LMS. High-definition 2D video and images were captured to enhance the teaching content. Faces of personnel and other identifiable traits were detected and blurred automatically using the Blace software tool. The DS's knowledge transfer component was developed using the eLearning tool Adobe Captivate and is structured around a tour of the facility.

III. RESULTS AND DISCUSSION

The Digital Slaughterhouse has been well received by students and teaching staff; it complements live visits, provides consistent teaching content, and supports the development of day-one competencies set by the European Association of Establishments for Veterinary Education and the

RCVS. The principles of desensitization, which involve gradually exposing individuals to anxiety-inducing stimuli in a controlled manner, have been well-established in psychology. Virtual simulations can be a valuable tool for implementing desensitization techniques by allowing individuals to experience and confront anxiety-provoking situations in a safe and controlled environment (1). For example, Kourtesis P. et al.(2) explore virtual reality for teaching training and higher education settings, discussing how VR simulations can provide opportunities to practice in difficult or challenging environments. Research in clinical psychology has demonstrated the efficacy of VR exposure therapy in treating phobias, anxiety disorders, and post-traumatic stress disorder (3). Other studies in various health education backgrounds support the use of VR as a way to recreate a realistic training environment that provides exposure to a challenging experience in a controlled environment (4, 5, 6,). Multiple pieces of evidence illustrate the effective design of multimedia learning material, emphasizing the importance of presenting information in multiple formats to cater to diverse learning styles and optimize learning outcomes (7,8,9).

Regarding limitations, virtual reality hardware, such as headsets and controllers, can be expensive, making it challenging for educational institutions with limited budgets to afford VR equipment. This cost barrier may prevent the widespread adoption of VR technology in some schools. Newer headsets are becoming more affordable. In addition, while VR can potentially enhance learning experiences, integrating VR into the curriculum effectively requires training and support for teachers. Lastly, during the VR experiences, motion sickness is common for users who are sensitive to virtual motion or have a pre-existing vestibular issue.

IV. CONCLUSION

The DS generated in this project can play an essential role in helping the students understand slaughterhouse practices. This VR aims to prepare the students mentally in a controlled environment to allow them to function better when facing the actual experience. The eLearning WebApp also enables them to learn, reflect, and/or review food safety and animal welfare concepts. Future steps could include adding some interaction in the VR environment to increase engagement, discovering ways to increase the usefulness of VR in the meat production industry, and determining the full effectiveness of how effective the use of the DS in education.

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Variation in Near Infra-red spectra collected from lamb carcases over multiple kills

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I. INTRODUCTION

As lamb carcases in Australia are not cut prior to bone out, there is a limited ability to sort carcases based on meat and eating quality traits. Consequently, there has been much research in the development of rapid, non-invasive tools such as Near Infra-red (NIR) spectroscopy. However, the results of such studies have demonstrated variation between predictive outcomes over multiple data collections [1]. Initial research has demonstrated factors such as pH decline can influence the NIR spectra [2], yet numbers in the study were limited and no other traits were measured. Therefore, a larger study has been conducted to assess the variation in spectra between data collection periods and its relationship with meat quality traits.

II. MATERIALS AND METHODS

At 24 hr post-mortem, the left *M. longissimus lumborum* (LL) was collected from 746 Merino lamb carcases in 7 kills. NIR spectroscopic measurements were conducted using an ASD® TerraSpec4 high resolution spectrometer with the ASD® contact probe [3]. Carcase data and meat quality traits including hot carcase weight (HCW), cold carcase weight (CCW), GR tissue depth, fat over the 5th rib, fat over C site, eye muscle depth (EMD), eye muscle length (EML), pH decline, pH at 24h, temperature at pH6, pH at 18°C, fresh colour, retail display colour, yield, ultimate pH (pHu) and shear force were also measured [4]. Principal Component Analysis was completed on raw spectra and spectra pre-processed via continuum correction and the first 2 scores were saved and regressed against all carcase and meat quality traits. Differences were noted as significant where the P value was <0.05.

III. RESULTS AND DISCUSSION

Although peaks of the spectra were similar between kills, there was a difference observed in the net absorbance of NIR spectra between kills (Fig 1) which was reflected by the PC scores. Consequently, PC scores were clustered based on kill, an effect which was amplified when spectra were pre-processed. Data for two kills (1 and 5) was excluded due to missing data.

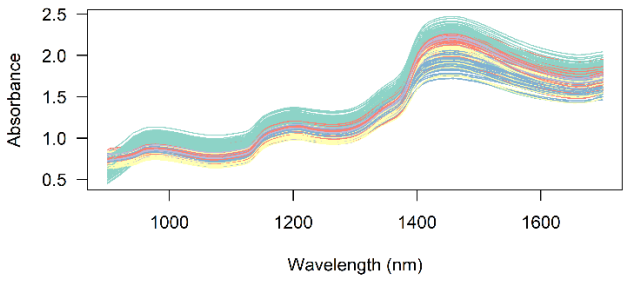
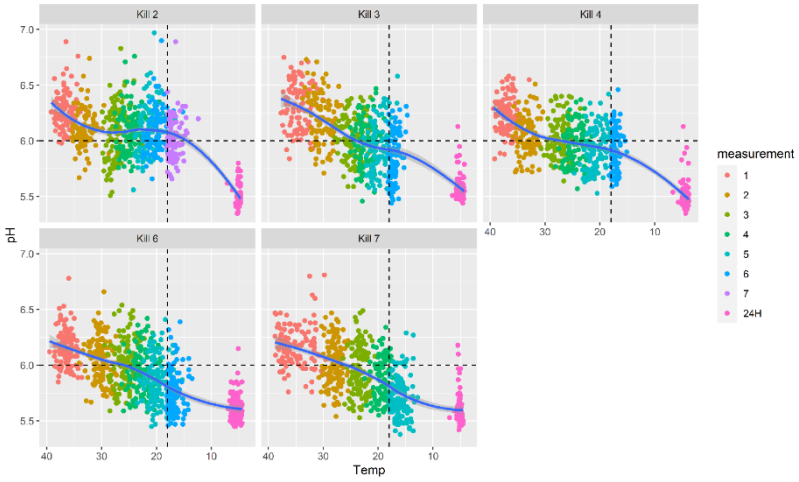


Figure 1. The average NIR spectra for each carcase measured.



Models to assess whether this variation was associated with any meat quality traits demonstrated that HCW, GR tissue depth, rib 5 fat, CCW, EMD, Fat at the C site, pH and temperature measured throughout the pH decline, retail colour traits and yield weights had a significant association with the PC scores 1 and 2 of both the raw and pre-processed spectra.

Figure 2. The pH and temperature decline for each kill.

Given the significance of pH and temperature measures and the indicators of muscularity and fatness which affect chilling rate, this results agree with previous research which suggested metabolic changes and temperature during the early post mortem period affect the NIR spectra [2]. This is supported by the pH decline which varied for kill 2 (Fig 2).

As this study also suggests a link between spectra variation and retail colour, it is hypothesised that the structural and biochemical changes which happen during rigor result in the shrinkage of the myofibril forcing water out of the myofibrillar structure and altering the light properties of the meat [5]. This could account for in the variation in the overall absorbance of the spectra and consequent clustering of PCA scores.

IV. CONCLUSION

This study demonstrated spectral variation is evident between kills and is associated with meat quality and carcase traits. Given many of the traits which were significantly associated are related to the pH/Temp decline, it is hypothesised that metabolic changes which occur during the early post-mortem period have an impact on the overall absorbance of NIR spectra.

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CAN THREE-DIMENSIONAL MODELS OF PIG CARCASSES PREDICT CARCASS WEIGHT AND TOTAL SALEABLE MEAT?

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I. INTRODUCTION

In 2023, pork emerged as the most consumed animal protein worldwide, and its production and consumption have been increasing since then [1]. Therefore, it is important to determine the carcass weight and total saleable or total lean meat yield with high precision for producers' remuneration. In commercial abattoirs, the weight of the carcass is determined by a scale positioned on the rails, but most do not collect information on the yield of the cuts [2]. Given these challenges, some technologies have been studied and used to facilitate the processes throughout the meat supply chain. The three-dimensional (3D) images stand out to provide a larger set of information, such as volume, area, perimeter, coordinates, and vectors. There are already studies evaluating 3D models for different species in the meat industry, such as lambs [3], beef cattle [4, 5], and swine [2, 6] but they are still scarce. Therefore, the objective of this study was to analyze the capability of a handheld 3D scanner to estimate the total volume of carcasses and predict the total saleable meat.

II. MATERIALS AND METHODS

In this study, 39 right half-carcasses of 5-month-old male swine from the Camborough x AGPIC337 (PIC Agroceres) cross were used. Hot carcass weight and 3D images were obtained before the chilling process, whereas the saleable meat yield was obtained after 24h of chilling. To obtain the 3D images, the right half-carcasses were fixed by a hook at a consistent position and height and manually scanned using a portable scanner (Artec Leo). The Artec Leo features automatic light adjustment, eliminating the need for additional lighting adjustments in the room. The digitized images were then imported into Artec Studio 17 software to generate the 3D models. With the final carcass 3D models, the carcass volume was recorded. The collected data were analyzed in RStudio software to investigate the relationship between carcass weight and carcass volume through simple linear regression. To determine the most relevant variable, prediction models were initially developed using carcass volume, hot carcass weight, and saleable meat weight. The regularized regression models chosen were Ridge, Elastic Net, and Lasso, from lowest penalty to highest, respectively. The most accurate and precise model was then subjected to linear regression analysis.

III. RESULTS AND DISCUSSION

The results suggest that the carcass volume of the 3D model is statistically significant ($P \leq 0.05$) to predict the total carcass weight. Therefore, it is possible to predict total carcass weight online with 3D volume using simple regression analyses. The adjustment of equation and error metrics showed that the equation generated is precise and accurate (Table 1).

Table 1 – Simple linear regression analyses to predict the total carcass weight and total saleable meat, using the total volume of the carcass obtained from the 3D model.

Volume	Intercept	β1	R^2	*P*-value	RMSE	MAE
Total carcass weight	4.8628	0.9192	0.9329	<0.001	0.7625	0.6803
Total saleable meat	10.0592	0.6478	0.4805	<0.001	0.9134	1.178

To predict total saleable meat, there was no gain by adding the hot carcass weight variable to the equation (Table 2). The models presented in Table 2 are used in larger databases to get a better fit in the model with a smaller number of variables without overfitting [7], but in this case, it was used to highlight the weight of the variables in the model. The penalization in the Lasso reduces variables judged irrelevant for

the model, making it simpler. Thus, Lasso exhibited the best fit among the models, with no significant differences observed among them, highlighting the importance of 3D model data. Once the variable has been selected, the prediction equation for total saleable meat (Table 1) performed well with low mean absolute error (MAE = 1.178). The moderate R^2 indicates that variable total carcass volume explains 48% of the total saleable meat, however, there is space for improvements.

Table 2 – Regularized regression models for variable selection, using carcass volume and carcass weight in the prediction of total saleable meat.

Total saleable meat (Y)	R^2	RMSE	MAE
RIDGE[1]	0.4782	1.1825	0.9178
ELASTIC NET[2]	0.4805	1.1802	0.9161
LASSO[3]	0.4805	1.1797	0.9158

[1]Y= 12.05543+0.02662×hot carcass weight+0.58297×carcass volume; [2]Y= 12.22485+0.60668×carcass volume; [3]Y= 11.96443+0.61163×carcass volume.

IV. CONCLUSION

The use of 3D models to predict total carcass weight and total saleable meat is possible, as the results presented are promising. More studies and more data are needed to improve the use of this technology, which will be of great benefit to the industry and to the farmers.

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Influence of Cutting Orientation and Aging Time on Juiciness of Different Beef Cuts

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I. INTRODUCTION

The succulence of meat is a crucial sensory aspect that directly impacts consumers' perception of quality. According to Smith et al. (2008), the direction of the cut significantly influences the juiciness of the meat. Cutting with the grain allows the fibers to shorten less during chewing, resulting in a softer and juicier texture. On the other hand, cutting against the grain may break the fibers, releasing more juices and enhancing the perception of juiciness. Meat aging also influences its juiciness. Smith et al. (2018) observed that aging for a period of 14 days resulted in a significant increase in meat succulence, attributed to the breakdown of muscle fibers and juice retention during the process.

Therefore, selecting appropriate orientation for cutting the meat and conducting effective aging are crucial to ensuring a satisfactory sensory experience. This study aimed to investigate the influence of cutting orientation on the succulence of different beef cuts (Longissimus thoracis and Biceps Femoris) during various aging periods.

II. MATERIALS AND METHODS

Three pieces of Longissimus thoracis and three pieces of Biceps Femoris were collected from the slaughterhouse of the Faculty of Animal Science and Food Engineering / University of Sao Paulo – FZEA/USP, located in Pirassununga, Sao Paulo. The samples were halved and subdivided according to maturation times, separating them for subsequent analyses. One half was intended for cuts parallel to the orientation of muscle fibers, while the other half was reserved for perpendicular cuts, against the fiber orientation. All pieces were labeled, vacuum-packed, and stored at 4°C until the predetermined maturation times (days 0, 7, and 14), and then frozen at -18°C.

The assessment of juiciness of the different cuts was performed through the analysis of weight loss during cooking. The pieces were cut into steaks with a thickness of 1 inch, resulting in a total of five steaks per piece, both for parallel and perpendicular treatments. Subsequently, the steaks were arranged in identified trays and individually weighed. The steaks were baked in an electric oven at 180°C until the geometric center reached a temperature of 70°C and then cooled at room temperature until 23°C. Subsequently, all exuded liquid from the trays was removed, and the samples were weighed again to evaluate the total weight loss during cooking. For the statistical analysis, a factorial experiment was conducted, in which the factors evaluated were: cut type (Longissimus thoracis and Biceps femoris), cutting orientation (parallel and perpendicular to the fibers), and maturation time (0, 7, 14 days). Tukey's mean test was applied with significance level ($p<0.05$).

III. RESULTS AND DISCUSSION

We observed that both the Longissimus thoracis and the Biceps Femoris showed a significant increase in the amount of exudate loss over maturation time. This is evidenced by higher weight loss during cooking on days 7 and 14 compared to day 0, in both cutting orientations (Table 1). Having day 0 as the day with the lowest amount of exuded liquid, as the fibers are still intact within the muscle, able to retain a greater amount of water. The lower water retention capacity of the meat implies losses of nutritional value due to the exudate released; when this capacity is reduced, significant nutrient losses occur due to the released exudate. This results in drier and less tender meat.

Table 1 - Cooking Loss Results by Fixing the Maturation Day

Day	Longissimus thoracis Parallel	Longissimus thoracis Perpendicular	Biceps femoris Parallel	Biceps femoris Perpendicular
0	62.41 b	19.32 b	29.96 b	25.16 c
7	97.23 a	83.65 a	130.00 a	187.33 a
14	94.00 a	72.59 a	134.93 a	110.28 b

Different letters in each column differ from each other by Tukey's test ($p<0.05$).

When comparing different cuts from the same muscle (Table 2), it is noticeable that cuts made perpendicular to the muscle fibers exhibited greater liquid retention during cooking compared to parallel cuts. This is consistent with the findings from Cross et al. (1978), which also emphasized the influence of cutting orientation on meat succulence, indicating that cuts made perpendicular to the muscle fibers result in a greater release of fluids during chewing, contributing to an immediate perception of succulence.

Table 2 - Cooking Loss Result by setting the cut orientation.

Cut	Longissimus thoracis Day 0	Longissimus thoracis Day 7	Longissimus thoracis Day 14	Biceps femoris Day 0	Biceps femoris Day 7	Biceps femoris Day 14
Parallel	62.41 a	97.23 a	94.00 a	29.96 a	130.00 b	134.93 a
Perpendicular	19.32 b	83.65 a	72.59 a	25.16 a	187.33 a	110.28 a

Different letters in each column differ from each other by Tukey's test ($p<0.05$).

On the other hand, parallel cuts tend to retain less liquid, resulting in a less pronounced succulence sensation. Bouton (1971) observed that cuts made perpendicular to the muscle fibers tend to retain more liquid throughout the cooking process, resulting in a prolonged sensation of succulence, while parallel cuts may exhibit a more pronounced initial succulence due to rapid fluid release. These findings underscore the importance not only of cutting orientation but also of fluid release dynamics in the overall meat succulence experience, suggesting that cutting orientation within the same piece of meat can directly influence the consumer's sensory experience, with cuts perpendicular to the muscle fibers potentially providing a juicier meat.

IV. CONCLUSION

The findings suggest that longer maturation periods result in greater weight loss, leading to reduced liquid retention and resulting in less succulent meat. Additionally, the orientation of the cut also plays a crucial role, with perpendicular cuts demonstrating superior succulence compared to parallel cuts. These results underscore the importance of carefully considering both maturation time and cutting orientation in meat preparation to achieve the desired sensory experience.

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CARCASS LEAN MEAT PERCENTAGE DETERMINED WITH COMPUTED TOMOGRAPHY IMAGES OF LIVE PIGS AND CARCASSES

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I. INTRODUCTION

Carcass lean meat percentage (LMP) is an important carcass grading parameter used to determine the carcass value and, in the EU, its determination is compulsory. At the abattoir, on line devices are used for this purpose which need to be previously calibrated. Calibration can be performed either by dissecting the full carcass, which is very time consuming and devalues the carcass, or by using computed tomography (CT) equipment [1]. The determination of LMP can also be performed in live pigs, either with CT or Ultrasounds [2,3].
The aim of this work is to determine the carcass LMP, lean and fat content from CT images of live pigs (with and without viscera) and carcasses.

II. MATERIALS AND METHODS

Nineteen pigs from different genetics and sexes (body weight = 110.5±8.66 kg) were fully CT scanned with the device Philips Brilliance 16, taking helical 3 mm-thick images every 3 mm (pitch 0.938), at 120kV, 200mA, field of view (FOV) 500 mm, 512 x 512 matrix and collimation 16x1.5. Pigs were previously anaesthetized with azaperone (0.10 mL/kg) and ketamine (0.06 mL/kg). After scanning pigs were slaughtered at IRTA abattoir. After 24 h of cooling, the left half carcasses, without head, foot and hand but with cheek were also CT scanned. Additionally, 20 carcasses from pigs from different genetics and sexes were CT scanned. The final average half carcass weight was 44.5±5.12 kg.
After scanning, carcasses were cut and fully dissected to obtain its weight of the lean and fat tissues. Then, the LMP was calculated according to EU legislation [1].
From CT images of live pigs, the viscera and internal organs were manually removed with the software VisualPork. From images of the live pigs with viscera and without viscera and from carcasses images, the volume associated with each Hounsfield (HU) value was determined using the image thickness, matrix size and FOV values [3]. In live pig images, the volume between HU values -149 and -1, 0 and 140, 141 and 499, 500 and 999, 1000 and 1499, 1500 and 2000 and between 141 and 2000 were calculated. Additionally, in carcass images, the volume between HU values 0 and 120 was calculated and divided by EU reference carcass weight to obtain the LMPCT variable.
Regression equations were obtained with SAS software (v. 9.4) for the prediction of carcass LMP, lean weight and fat weight using the volumes as predictors. For live animal images, stepwise selection was used to select the most suitable predictor.

III. RESULTS AND DISCUSSION

Average LMP, lean weight and fat weight obtained by dissection were 58.7±4.04 %, 26.1±3.38 kg and 9.5±2.48 kg, respectively. The prediction equations for each parameter and its goodness of fit are presented in Table 1. Considering live pigs, the error of prediction for all the parameters was lower when viscera are removed from the images. Removing the viscera from the images is very time consuming and it is operator dependent. Thus, it is worthwhile to consider the improvement in error and the cost to remove the viscera from the images. Moreover, the lowest relative error (CV) was always for the LMP and the lean content and the highest for the fat content. The RMSEP for LMP prediction from carcass images was 0.95%, which was slightly higher than those reported in a previous

trials using similar methodology (0.81%) [4] or a different one using much more predictors and simplified dissection (0.82%) [3].

Table 1 – Regression equations to determine carcass lean meat percentages (LMP) from computed tomography images of live pigs, live pigs without viscera and left half carcasses.

Images of:	n	Predicted carcass trait:	Prediction equation[b]	RMSEP	CV (%)	R^2
Live pigs	18[a]	LMP (%)	54.68513 – 0.44395 * sum-149-1 + 0.52921 * sum0+140 – 2.55891 * sum141+499	1.29	2.16	0.955
Live pigs	18[a]	Lean (g)	8391.77601 + 838.08894 * sum0+140 – 1587.95822 * sum141+499	1068.57	2.06	0.970
Live pigs	18[a]	Fat (g)	1086.86129 + 665.51737 * sum-149-1 - 72.48307 * sum0+140	862.66	5.05	0.972
Live pigs without viscera	18[a]	LMP (%)	56.52898 – 0.45261 * sum-149-1 + 0.47771 * sum0+140 – 1.53063 * sum141+2000	1.11	1.86	0.966
Live pigs without viscera	18[a]	Lean (g)	-6.21985 + 129.81391 * sum-149-1 + 911.55643 * sum0+140	983.54	1.90	0.977
Live pigs without viscera	17[a]	Fat (g)	-3376.69834 + 775.06682 * sum-149-1 + 529.27463 * sum141+499	692.22	4.05	0.983
Carcasses	39	LMP (%)	7.30207 + 0.90332 * LMPCT	0.950	1.62	0.949
Carcasses[c]	38[a]	Lean (g)	-11611 + 360.95302 * sum-149-1 +830.59708 * sum0+140 +226.79518 * LMPCT	368.384	1.41	0.990
Carcasses[c]	38[a]	Fat (g)	437.96287 + 887.17979 * sum-149-1	304.350	3.22	0.986

[a] one/two carcass removed as outliers; the sums used as predictors (volumes) were in dm^3; [b] sumXY: volume associated to Hounsfield values between X and Y. [c] lean and fat of half carcass. RMSEP: root mean squared error of prediction; CV: 100*RMSEP/Mean; R^2 Coefficient of determination; LMPCT=sum0+120/carcass weight.

IV. CONCLUSION

In the conditions of the present trial, it is possible to predict the carcass LMP with a good error of prediction both, from CT images of live animals (with and without viscera) and from carcass, the last one being the most precise. The prediction of the lean content is more precise than those of the fat content in all the cases when images from live pigs or images from carcasses were used.

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CAMERAS AND AI TO ENHANCE THE MEAT INSPECTION PROCESS

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I. INTRODUCTION

The augmentation of visual meat inspection garners increasing attention due to its crucial role in ensuring food safety [1]. Currently, meat inspection is performed entirely by trained personnel. However, it can be argued that the inspection would be performed with greater accuracy and precision if computer vision systems (CVS) could contribute to the official meat inspection (OI) compared to not using this technology. The high intra- and inter-rater variation between human inspectors [2] could be minimized as OI may be conducted more consistently and be specifically targeted at detecting small contaminations on the large surfaces. Introduction of the new EU legislation on official controls in food production allows the use of CVSs as complementary tools in meat inspection. Therefore, in Denmark, an experimental equipment has been installed at one pig and two beef slaughter lines to augment the process of meat inspection, Figure 1. The CVS uses a trained Convolutional Neural Network (CNN) to detect plausible fecal contaminations of a size of approx. 0.5x0.5mm to 2x2mm depending on the camera set-up. This recent work evaluates the application of CVS in detecting fecal contamination on pig carcasses just before the post-mortem inspection phase at the slaughter line (Figure 1 left). The aim was to explore statistical techniques to evaluate the sensitivity (Se) and specificity (Sp) of CVS compared to OI in the absence of a true gold standard evaluation.

Figure 1. Pictures of the various computer vision systems (CVS)
Left: The CVS viewed from above the pig slaughter line, 4 towers each with 3 RGB+NIR cameras take 24 pictures of each half carcass side.
Middle: The 2-cam system imaging beef hind legs after dehiding points out contamination for operator steam vacuuming.
Right: The BCC-3^{TM} [3] end-of-line equipped with 3 high resolution RGB cameras in each of the 4 corner towers imaging each half carcass.

II. MATERIALS AND METHODS

Data subsets originating from 15 representative normal production days of a slaughter line, processing at 428 carcasses/h, were collected for analysis and included a total of 71,298 pigs [4]. Carcasses with major slaughter defects were omitted as they were unsuitable, by definition, for inspection by either the OI or CVS [5].

The OI fecal findings were recorded on the main line inspection platform by three OI inspectors. OI observations were recorded by rotating inspectors according to internal guidelines, with inspectors being able to assign more than 35 different OI carcass remarks, albeit a maximum of 4 per carcass [5].
The analysis method used descriptive statistics, agreement calculations between CVS and OI and latent class modelling [6] to estimate the Se and Sp of both the methods.

III. RESULTS AND DISCUSSION

Through the application of latent class modelling, the Se and Sp of the CVS system were estimated at 31% and 97%, respectively, in contrast to the OI's 20% Se and 99% Sp (Table 1). CVS was better at detecting fecal contaminated carcasses with a Se of 31% versus 20% for the OI. Contrarily, the OI had, as expected, a near-perfect Sp of 99% versus 97% for CVS, demonstrating that both systems were adept at classifying carcasses devoid of fecal contamination, albeit with a slight edge to the official inspection. The results demonstrate the comparative strengths and limitations of the CVS and traditional OI. The CVS closely aligns with public health objectives by prioritizing the detection of contaminants to enhance food safety by ensuring contamination is identified, albeit at a risk of more false positives and higher operational cost due to the need for technology investment and its increased sensitivity. Conversely, the OI approach offers an efficient solution for food business operators, with high specificity effectively reducing false positives and associated costs, yet with a potential risk of missing some contaminated carcasses due to lower sensitivity. The combination of CVS and OI seems a way to increase the overall performance. The results highlight the possibility of using latent class modeling to estimate Se and Sp, though the specific values have not been validated and might be influenced by varying slaughter processes during the test.

Table 1 – The Se and Sp of both CVS and the OI as estimated by the latent class model. The latent class model's 95% confidence interval is shown in brackets. Also included are the results of using OI or CVS, respectively, as the gold standard.

Evaluation-group	CVS		Official Inspection (OI)	
	Sensitivity	Specificity	Sensitivity	Specificity
Latent class model	31% [27%-38%]	97% [95%-99%]	20% [14%-26%]	99% [99%-100%]
CVS-Gold standard/ Official inspection	29%	93%	-	-
OI-Gold standard / VISION	-	-	30%	97%

IV. CONCLUSION

The sensitivity and specificity of the CVS system were estimated at 31% and 97%, respectively, in contrast to the OI's 20% Se and 99% Sp. At present, the utilization of CVS technology as an aid to enhance detection of contaminations, subject to verification by OI, emerges as a feasible strategy. However, CVS CNN modelling is being iteratively improved with more data and better methods giving promising reductions in false positives, making the technology increasingly relevant.

ACKNOWLEDGEMENTS

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ESTIMATING BEEF CARCASS COMPOSITION USING 3D IMAGE MODELS

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I. INTRODUCTION

Image-based systems for automatic carcass classification are increasingly being studied and proposed in the literature with the aim of increasing accuracy and avoiding human subjectivity [1]. A 3D beef carcass model can be analyzed using innovative technologies such as video image analysis (VIA) and machine learning algorithms to predict live weight, carcass characteristics, and meat yield accurately [2]. To achieve the yield of the cuts and their composition, it is necessary to apply an exhaustive deboning method. This method is usually carried out manually, making it a slow process that requires hours of work, being costly and subject to notable biases associated with the dexterity of butchers. Therefore, it is important to develop a simple, fast, and accurate method to replace traditional approaches in the business environment [3]. It is estimated that through a computer vision system based on three-dimensional digital image analysis we can improve the quality of the beef industry by automating and standardizing the process of evaluating carcass quality and tissue composition. Therefore, the objective of this research was to evaluate the relationship between the volumetric measurement of digital images with total carcass weight and the yield of commercial cuts of the bovine carcass.

II. MATERIALS AND METHODS

Data were collected from 36 young Nellore males (18 bulls and 18 steers), receiving maintenance (n = 8), high (n = 14), or low (n = 14) concentrate diets. The treatments were selected to maximize the range in hot carcass weight and fat score. The animals were slaughtered at the Slaughterhouse of the Universidade Federal de Viçosa (UFV), following the brazilian standards of the Technical Regulation for Pre-Slaughter Management and Humane Slaughter. After harvest, the half-carcasses were weighed to obtain the hot carcass weight (HCW). Subsequently, cold carcass weight (CCW) was obtained after chilling period of 24h at 4ºC. The left half-carcasses were scanned before being stored in the cold chamber and after chilling, obtaining 3D models of hot and cold carcasses. The 3D scanner used was the Artec Leo, configured with a 3D reconstruction rate of 30 FPS (frames per second) and a 3D resolution of 0.2mm, with an accuracy of 0.1mm. The 3D images processing was performed using the Artec Studio 17 software, obtaining measurements of the biometric parameters of volume and area. After 3D scanning, the carcasses were sectioned into anatomical regions that constitute commercial meat sections. The commercial cuts were obtained and weighed according to the routine deboning adopted by the UFV slaughterhouse. The evaluation of the 3D model for estimating carcass weight and cuts yield in comparison with the traditional deboning was by means of linear regression testing the slope and intercept parameters. Precision was assessed by the coefficient of determination (R^2) and accuracy by mean absolute error (MAE). Statistical analyses were performed in SAS (Institute Inc., Cary, NC, USA).

III. RESULTS AND DISCUSSION

Figures 1 and 2 show the equations for predicting the carcass composition using 3D imaging models. It can be observed in both graphs that the coefficient of determination was high, establishing a high correlation for both the weight of the hot carcass and the yield of cuts in relation to the volume extracted from the 3D image models generated from the scanning of the hot carcasses. The P values for the slope coefficients in both regression equations were statistically significant ($p<.0001$).

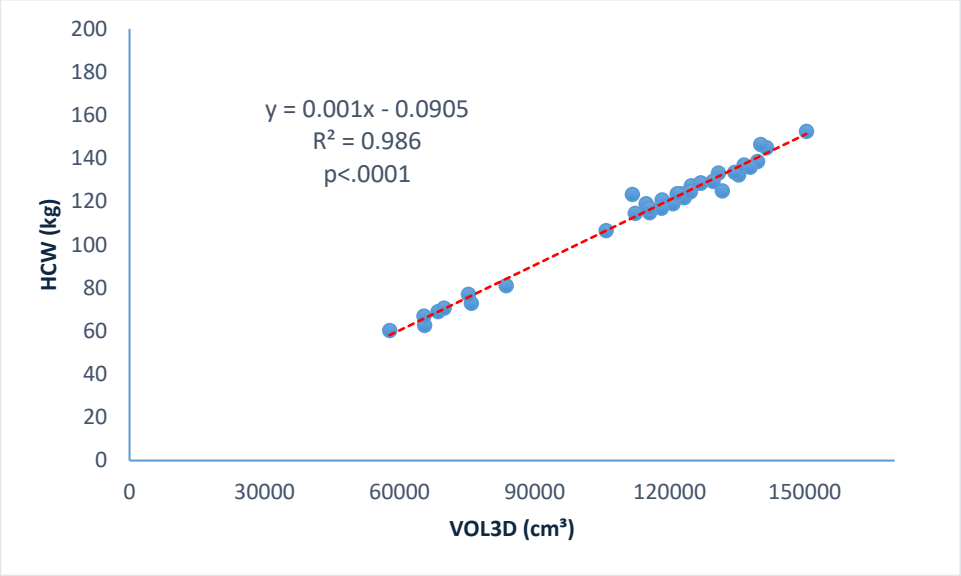

Figure 1. Correlation between hot carcass weight and volume obtained from the 3D model.

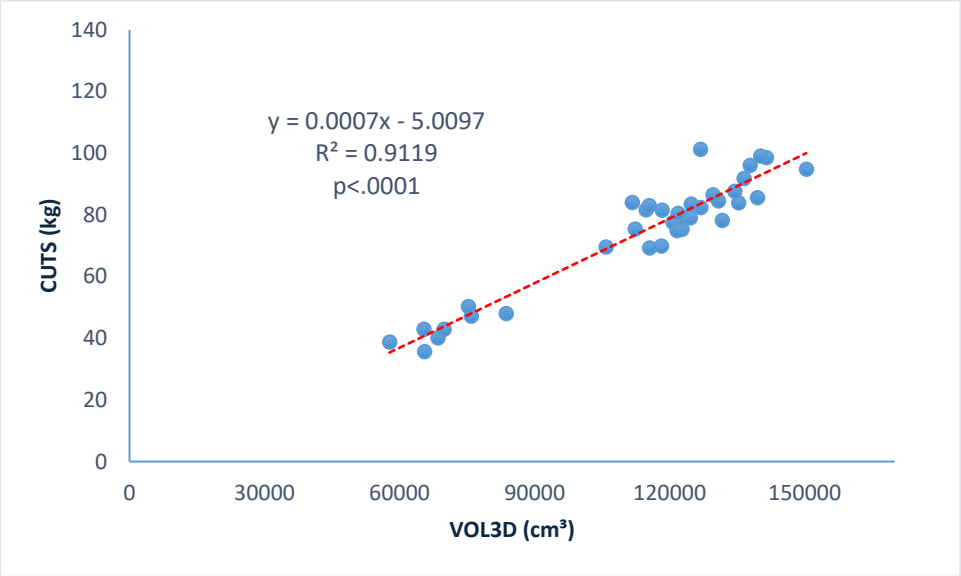

Figure 2. Correlation between commercial cuts yield obtained from the carcass and volume obtained from the 3D model.

IV. CONCLUSION

The 3D images can be used to predict total weight and carcass commercial cut yields. However, more studies are necessary since our equations are based on Nellore bulls and steers and low sample size. This technology can be makes the carcass evaluation process more automated, allowing many potential improvements in the meat industry, offering new perspectives for the evaluation of the superior quality beef carcasses.

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BEEF TENDERNESS BY NMR: DETERMINATION IN A NON-DESTRUCTIVE WAY

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I. INTRODUCTION

Tenderness is one of the most desired requirements by producers, consumers, and the meat industry, and this has become more evident every day with the year-over-year increase in supply and demand for meat from *Bos taurus* breeds and/or crosses with *Bos indicus* cattle predominant in Brazil.
The beef industry requires methods for analyzing the quality of this product that are non-destructive and work in real time, in order to improve production efficiently and still meet consumer expectations [1]. The most common methods for evaluating meat tenderness are mechanical, destructive and time-consuming, such as the Warner-Bratzler Shear Force (WBSF) and the Slice Shear Force techniques, or even sensory evaluation.
Nuclear magnetic resonance (NMR) is a non-invasive, fast and accurate method and has been used to analyze meat quality [2]–[4]. Therefore, the objective of this study was to develop a piece of equipment (Figure 1a) and an NMR method capable of quickly and non-destructively determining the tenderness of packaged meat.

II. MATERIALS AND METHODS

Low-field NMR equipment capable of analyzing meat samples with maximum cross-sectional dimensions of 70 mm x 170 mm was developed. NMR signals were collected in duplicate from 750 samples of the *longissimus* muscle with a thickness of 2.5cm for the development of the equipment software and the NMR analysis method. These signals were correlated, using chemometrics, with the WBSF values [5] of the same samples. Afterwards, another 100 *longissimus* muscle samples were analyzed in duplicate and a PLS (Partial Least Squares) model was developed to predict meat tenderness based on NMR signals. The sample set was divided into a calibration and external validation set in proportions of 80 and 20%, respectively. The calibration was done with leave-one-out cross validation.

III. RESULTS AND DISCUSSION

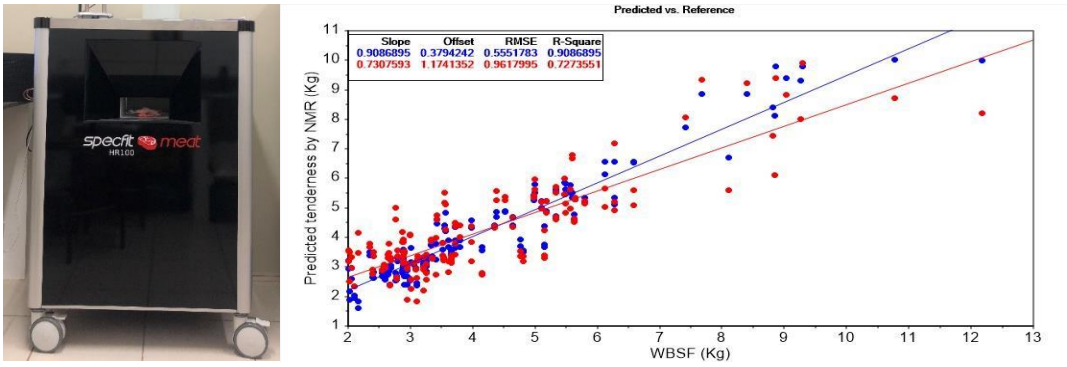

Figure 1. **a:** NMR equipment developed; **b:** PLS correlation plot using NMR signals and WBSF (blue points: calibration; red points: cross-validation).

The tenderness values predicted by NMR and determined by WBSF, as well as the parameters for evaluating the performance of the PLS model (errors and coefficients of determination, R^2) are presented in figure 1. Models with R^2 values above 0.70 show high correlation between techniques (Figure 1b), in addition, we believe that the average error is within an acceptable limit for the industry. In the external validation stage (Table 1), the tenderness results obtained by the PLS model were close to those determined by WBSF. Tenderness determination in a non-destructive way brings great benefits to the

beef industry and consumers, as it makes it possible to market and sell the product with tenderness information on the packaging.

Table 1 – Meat tenderness results as predicted by NMR technique and determined through WBSF testing.

Sample	Predict RMN	WBSF	Standard deviation	Sample	Predict RMN	WBSF	Standard deviation
1	3.74	3.34	0.20	21	2.99	2.89	0.05
2	3.71	3.34	0.18	22	3.49	2.89	0.30
3	3.63	3.72	0.05	23	3.54	3.66	0.06
4	3.88	3.72	0.08	24	3.57	3.66	0.05
5	2.96	2.06	0.45	25	3.00	3.26	0.13
6	2.59	2.06	0.26	26	2.84	3.26	0.21
7	2.39	2.68	0.15	27	3.79	3.53	0.13
8	2.39	2.68	0.15	28	3.57	3.53	0.02
9	3.43	3.66	0.11	29	3.34	3.35	0.01
10	3.40	3.66	0.13	30	3.32	3.35	0.02
11	3.02	2.89	0.06	31	3.28	3.26	0.01
12	3.02	2.89	0.06	32	2.67	2.78	0.06
13	3.58	2.89	0.34	33	2.56	2.78	0.11
14	3.27	2.89	0.19	34	3.18	3.26	0.04
15	3.25	3.22	0.02	35	3.66	4.16	0.25
16	3.07	3.22	0.08	36	3.56	4.16	0.30
17	2.87	2.95	0.04	37	3.48	2.36	0.56
18	3.07	2.95	0.06	38	3.39	2.36	0.51
19	3.31	2.80	0.25	39	2.78	2.55	0.11
20	3.18	2.80	0.19	40	2.68	2.55	0.06

IV. CONCLUSION

The results prove the viability of using the developed equipment in a non-destructive way to predict beef tenderness, using a developed method of NMR that shows high correlation and low average error.

ACKNOWLEDGEMENTS

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EFFECT OF SPACE REDUCTION ON THE COOLING RATE OF PIG CARCASSES IN STATIC COOLING CHAMBERS

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I. INTRODUCTION

The cooling of pig carcasses after slaughter is a fundamental process to maintain the microbiological quality of the product. For this purpose, the process must occur in a way that ensures the carcasses reach a maximum internal temperature of 7 °C within a period of 12 to 24 hours, to proceed to carcass cutting [1]. In industrial settings, carcasses are suspended in hangers for colling in cold chambers. The spacing between hangers must be at least 0.33 cm, allowing for three carcasses per linear meter, following the Brazilian regulations [1]. Cold chamber capacity stands as a limiting factor in increasing pig carcass processing at slaughterhouses. Hence, the increase of carcass density within chambers may enhance productivity. This study aimed to assess the impact of reducing the spacing between pig carcasses within static cooling chambers on cooling rate, pH and microbial counts of the carcasses.

II. MATERIALS AND METHODS

The study was conducted at a pork slaughterhouse located in the western region of the state of Paraná, Brazil. A total of 120 carcasses (average weight of 100 kg) were assessed in six cooling chambers (20 carcasses in each chamber). The carcasses underwent two treatments: TP, with a density of four carcasses per linear meter (0.25 cm spacing), and TC, with a standard density of three carcasses per linear meter (0.33 cm spacing). Within the chambers, the carcasses were uniformly positioned on the same track and region. The carcasses were monitored and temperature, measured hourly, pH, measured every two hours, and microbiological count, evaluated at 0, 4, and 12 hours, were assessed. The internal temperature was recorded using a thermometer (Hobo® U12, Massachusetts, USA), and pH was determined using a penetration pH meter (Testo 205, Titisee-Neustadt, Germany), both inserted at the geometric center of the ham. Microbiological samples were collected on ham by swabbing a disposable mold of 100 cm². The mesophilic aerobic count was conducted on PCA agar incubated at 30±1 °C for 72 h, and the Enterobacteriaceae count was performed on Enterobacteriaceae Count Plate incubated at 37 °C ± 1 °C for 24 h. Results were expressed in log CFU/cm². Data analysis utilized the statistical model $Y = aX + bY + \varepsilon$, where X represented treatments (0.25 cm and 0.33 cm spacing) and Y represented evaluation times (0 to 12). Mean comparisons were performed using GLM for linear models, employing two-way ANOVA in a factorial arrangement, followed by Tukey's test ($P<0.05$). To analyze the carcass temperature decrease over time, a modified Newton model was applied, according to the equation: $T = a(1+k)\ t+b$, where T represented the temperature as a function of time t, (a + b) denoted the initial temperature provided by the model, k indicated the decay rate, and b signified the temperature after a prolonged period.

III. RESULTS AND DISCUSSION

The mean temperatures for hot carcasses, measured inside the ham at the cooling chamber entrance, were 38.22 °C in TP and 38.25 °C in TC, within the typical range for hot carcasses after slaughter, which typically ranges from 38 °C to 41 °C [2]. There was no interaction between treatment and time for carcass temperature ($P > 0.05$). Treatment had no effect; only time exhibited a significant impact on temperature ($P < 0.05$). The mean temperature decrease rate (k) was -0.189 °C (TP) and -0.182 °C (TC). There was a notable reduction in temperature over time, with hams beginning to stabilize around t 9 in TC and t 10 in TP. By t 12, mean temperatures were 6.80 °C in TP and 6.84 °C in TC ($P > 0.05$). For pH, there was likewise no observed interaction between effects ($P > 0.05$), with only time exhibiting a significant effect, resulting in decreasing values over the evaluation period. Initial pH values (t0) stood at 6.47 in TP and 6.47 in TC, while final values (t12)

were 5.94 in TP and 5.99 in TC (P > 0.05). Correspondingly, like other parameters, only time displayed an effect on both mesophilic aerobic and enterobacteria counts, showing a decline in treatments throughout the cooling period (Table 1). Mesophilic aerobic counts in pig carcasses exhibit variability across studies, with reported values ranging between 2.40 and 4.95 log CFU/cm² [3, 4, 5]. The recorded values generally align with the lower end of this range, but below the thresholds established by Brazilian legislation and the European Union (5 log CFU/cm²) [6, 7]. Enterobacteria counts were initially below 0.23 log CFU/cm² at t0, experiencing a significant reduction to 0.04-0.05 log CFU/cm² at t12, values below the standards set by Brazilian legislation (3 log CFU/cm²) and the European Union (5 CFU/cm²) for pig carcasses [6, 7].

Table 1 - Mesophilic aerobic and enterobacteria counts in ham of pig carcasses during cooling in static cold chamber.

Time (h)	Mesophilic aerobic (log UFC/cm²)		Enterobacteria (log UFC/cm²)	
	TP	TC	TP	TC
0	2,75 ± 0,95 ^{aA}	2,79 ± 0,86 ^{aA}	0,21 ± 0,40 ^{aA}	0,23 ± 0,43 ^{aA}
4	2,26 ± 0,80 ^{bA}	2,33 ± 0,66 ^{bA}	0,07 ± 0,23 ^{bA}	0,08 ± 0,22 ^{bA}
12	2,37 ± 0,93 ^{abA}	2,26 ± 0,64 ^{bA}	0,04 ± 0,15 ^{bA}	0,05 ± 0,16 ^{bA}

Means followed by the same lowercase letters in the same column do not present significant differences between times (P <0.05); means followed by the same uppercase letters in the same row do not present significant differences between treatments (P < 0.05). TP = protocol treatment; TC = control treatment. Mean ± standard deviation.

IV. CONCLUSION

Optimizing the occupancy rate to four pig carcasses per linear meter in the cooling chambers has demonstrated efficacy in lowering internal carcass temperature and managing microbial proliferation. Consequently, narrowing the spacing between pig carcasses, as observed in this study, can be adopted without adverse effects on cooling efficiency or carcass microbiological quality. This adaptation enables increased cooling capacity and higher pig carcass production volume.

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PREDICTING BEEF CARCASS ULTIMATE pH: OCULAR THERMOGRAPHY AND BLOOD PARAMETERS

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I. INTRODUCTION

Brazil holds a prominent position in global beef production and export, boasting a significant cattle population primarily composed of Zebu breeds, adapted to the tropical climate. Despite its economic importance, the industry faces challenges such as the occurrence of dark, firm, and dry (DFD) meat, attributed to pre-slaughter stress. DFD meat not only diminishes quality but also affects consumer satisfaction and shelf life. To address this issue, noninvasive methods for DFD meat identification, such as infrared thermography (IRT), have garnered interest due to their potential for swift and accurate assessment. Therefore, the aim of this study was to investigate the use of IRT as a tool to identify DFD meat based on ocular temperature.

II. MATERIALS AND METHODS

This study was conducted in a Federally Inspected commercial slaughterhouse in Brazil, adhering to the prevailing regulations [1]. A total of 113 male Nellore bulls (aged 22–38 months) were included, with no batch intermixing during transport and unloading. Eye temperatures were measured within the stunning box without interfering with industry standard procedures. Ocular IRT images were captured using a Flir T440 camera. Each animal's right eye was framed at a 90º angle from 60 cm. FLIR TOOLS® software analyzed the IRT images to determine minimum (IRTmin) and maximum (IRTmax) temperatures. The carcasses final pH (pHu) was measured twice, between the 12th and 13th ribs in the *longissimus thoracis muscle*, using a professional pH meter, Hanna HI98163® (Hanna Instruments Inc., Barueri, Brazil), 48 hours post-mortem.

Blood samples were collected from the animals during bleeding, stored in 10 mL BD Vacutainers® tubes, and analyzed in duplicate using commercial kits. Glucose, CK, LDH and lactate were assessed with the Dimension® Xpand Plus system and respective kits. Cortisol levels were measured in plasma using a commercial cortisol ELISA kit, with absorbance read at 450 nm on an iMark™ Microplate Absorbance Spectrophotometer (Bio-Rad Laboratories Inc.). Data analysis utilized Jamovi® software, with significance set at $P < 0.05$. Linear and multiple regression models were employed to assess relationships between ocular thermal temperatures, blood parameters, and carcass pHu. Day of collection and batch were treated as random effects, with other variables (lactate, glucose, CK, LDH, cortisol) as covariates. Cross-validation, randomly allocating 70% for calibration and 30% for prediction, validated the dataset. Model adequacy was assessed through metrics including R2, adjusted R2, RMSE, RPD, and ANOVA-derived P-value.

III. RESULTS AND DISCUSSION

Descriptive statistics revealed stable environmental conditions during data collection [2]. Regression analyses demonstrated the predictive capabilities of IRT, either alone or in conjunction with blood parameters, in estimating carcass pH (Table 1). The first line of each set contains only IRTmax as a pHu predictor. For the calibration dataset, the R^2 values range from 0.84 to 0.88. Higher R^2 values

indicate that a larger proportion of the variability in the dependent variable is explained by the independent variables. The predictors IRT max + Lactate and IRT max + Glucose + Lactate had the highest R^2 values at 0.88. For the predicted dataset, as shown in the bottom section of the Table, the models retained their predictive power, albeit with a slight reduction in R^2 values compared to the calibration phase. The R^2 values range from 0.67 to 0.87. IRT max + Glucose + Lactate has the highest R^2 on the predicted dataset at 0.87, and an impressive RPD value of 2.6, suggesting excellent predictive capability. The RMSEP values for these models were relatively low, ranging from 0.104 to 0.152, indicating good accuracy in predicting pHu during the calibration phase. In both training and test datasets, lower RMSE values indicate better model performance. The models seem to perform well on the training data, but there is a slight increase in error when applied to the test data, which is common.

Table 1. Linear and Multiple regression of ocular IRT and beef quality traits and blood parameters to predict pHu of Nellore beef carcasses.

Calibration predictors	R^2	R^2 adjusted	RMSEC		*P*
IRTmax	0.84	-	0.0841		< 0.001
IRTmax + Glucose	0.85	0.84	0.0843		<0.001
IRTmax + Lactate	0.88	0.87	0.0739		< 0.001
IRTmax + Glucose + Lactate	0.88	0.86	0.0746		< 0.001
Predicted	R^2	R^2 adjusted	RMSEP	RPD	*P*
IRTmax	0.67	-	0.152	1.8	< 0.001
IRTmax + Glucose	0.74	0.69	0.144	1.9	< 0.001
IRTmax + Lactate	0.82	0.78	0.117	2.3	< 0.001
IRTmax + Glucose + Lactate	0.87	0.82	0.104	2.6	< 0.001

IRT max = infrared thermography maximum. R^2 = determination coefficient. R^2 adjusted = determination coefficient adjusted. RMSEC = root mean square error of calibration. RMSEP = root mean square error of prediction. RPD = residual Prediction Deviation.

IV. CONCLUSION

The study suggests that ocular IRT image, complemented by specific blood parameters, can effectively predict carcass pH in Nellore beef. The integration of lactate and glucose enhances model accuracy, highlighting the potential utility of IRT for noninvasive quality control in the meat industry.

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PROTOCOLS EVALUATION FOR SERUM METABOLOMICS ANALYSIS IN BEEF CATTLE

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I. INTRODUCTION

Meat tenderness is traditional evaluated on meat after slaughter, however, strategies to improve the prediction of such trait during animals' life has a potential to improve the farmers and industry decisions on strategies of commerce and genetic selection. Since meat tenderness had been recently correlated with oxidative stress, apoptosis, mitochondria and energetic metabolism [1,2], analysing blood metabolites might provide a clue of those aspects and facilitate the prediction of tenderness in animals live. In this line of reasoning, quality and impact of metabolomics studies depend on the sample preparation method. For that, this study has been designed to compare four sample preparation methods for beef cattle serum samples by assessing the spectral quality, metabolite identification, reproducibility, speed and relative cost.

II. MATERIALS AND METHODS

Blood samples (10 ml) were collected from five steers, samples were centrifuged for 15 min at 3000 × *g* at 4 °C; the serum was transferred into eppendorf tubes. Three technical replicates were used for each sample preparation method (a total of 12 samples). Samples were prepared following the methods: metabolites extraction using methanol and chloroform (M+C); methanol, chloroform and acetone (M+C+A); filtering the samples in a 3 KDa filter (3 KDa); filtering the samples in a 2 µM filter (2 µM) [3]. Metabolomics was evaluated through nuclear magnetic resonance spectroscopy (^{1}H-NMR). NMR spectra were recorded at 298 K using a 14.1 T Bruker spectrometer (600 MHz for hydrogen frequency), equipped with a 5 mm TCI cryoprobe. Spectra were processed using Chenomx Software v 10.0. Data were analyzed using MetaboAnalyst 5.0 (www.metaboanalyst.ca), Venn diagram was accessing the web tool Jvenn (http://jvenn.toulouse.inra.fr/app/usermanual.html). The methods were evaluated based on the spectra quality, reproducibility, easily processing, speed and relative cost [3].

III. RESULTS AND DISCUSSION

The use of 2 µM filters did not yield high-quality spectra for serum samples, leading to the exclusion of this treatment from further analysis. Principal Component Analysis (PCA) revealed a distinct separation between samples processed with the 3 KDa filter and those treated with chemicals, while an overlap was observed between samples treated with M+C+A and M+C. Twenty-nine metabolites were identified in serum samples processed using chemical methods, whereas a greater number (47 metabolites) was detected when the 3 KDa filter was employed. The filter also resulted in higher metabolite concentrations and improved reproducibility.

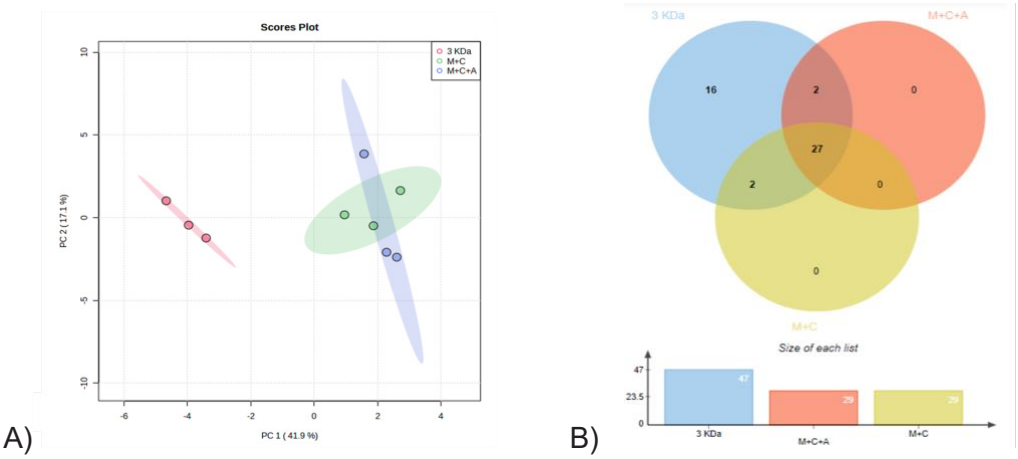

Figure 1. A) PCA scores plot of metabolites profile in serum according to different metabolites extraction methodology (red, 3 KDa; green, C+M; and dark blue, C+M+A). B) Venn diagrams of the number of extracted metabolites in cattle serum samples according to different metabolites extraction methodology (blue, 3 KDa; orange, M+C+A; and yellow, M+C).

Table 1 Qualitative scores of serum preparation according to different metabolites extraction methodology. Scores: 1 – poor; 2 – acceptable; 3 – excellent.

Method	Spectral quality	Metabolite's identification	Metabolite's quantification	Reproducibility	Speed	Relative cost	Total
M+C	3	2	2	2	1	3	13
M+C+A	3	2	2	2	1	3	13
2 µM	1	-	-	-	1	2	4
3 KDa	3	3	3	3	2	1	15

M+C: Methanol:chloroform (1:1, v/v); M+C+A: Methanol:chloroform:acetone (1:1:1, v/v/v); 3 KDa filters (Amicon Ultra -0.5, Merck Millipore Ltd. Brazil); and 2 µM filters (Analitica Scientific, São Paulo, Brazil). Adapted of Samuelsson et al. [4]

IV. CONCLUSION

The 2 µM filter is not suitable for removing macromolecules from serum samples. Conversely, the 3 KDa filter has been confirmed as a highly suitable method for preparing beef cattle serum samples for NMR-based metabolomics.

ACKNOWLEDGEMENTS

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METABOLOMIC FINGERPRINTING OF NELLORE CALVES WITH VARIATION IN BIRTH WEIGHT

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INTRODUCTION

Variation in the birth weight of beef cattle is generally explained by genetic and non-genetic fetal and maternal factors [1]. In this context, interactions between genes and environmental factors generate specific metabolic rates and/or metabolisms that may represent the phenotype of interest [2]. Metabolomics is an analytical tool used to evaluate metabolites, which in turn provides a better understanding of the metabolism that generates the phenotype [3]. Considering the differences in birth weight, it is believed that variations in the metabolism of these animals may exist. Therefore, the aim of this study was to evaluate whether differences in birth weight lead to changes in the calves metabolism.

MATERIALS AND METHODS

The experimental procedures were conducted in accordance with the Institutional Animal Care of the College of Animal Science and Food Engineering at the University of São Paulo (9249180123). A total of 100 male calves were separated into two treatments based on the average weight: high birth weight (HW, $\leq$ 35 kg) and low birth weight (LW, $\geq$ 43 kg). Blood samples and weight measurements were taken on day two after birth. Blood samples were taken through the jugular vein using a vacutainer tube of 10 mL, samples were centrifuged for 15 min at 2000 × g at 4°C. Serum was collected, and macromolecules were removed using 3 kDa filters (Amicon® Ultra - 0.5, Merck Millipore Ltd, Ireland) [4]. The spectra were obtained by nuclear magnetic resonance spectrometry (1H-NMR), exported to the online tool NMRProcFlow - version 1.4.24 (https://nmrprocflow.org/), processed, and divided into buckets of 0.05 ppm width, resulting in 111 buckets. Statistical analyses were performed using the web tool MetaboAnalyst 6.0 (http://www.metaboanalyst.ca/), employing principal component analysis (PCA) and Volcano plot.

RESULTS AND DISCUSSION

The PCA analysis showed similarity between the groups (Figure 1A). However, in the Volcano analysis (Figure 1B), calves born with higher weight had higher concentrations of lactate and histidine, and lower concentrations of 3-hydroxybutyrate and glucose compared to calves in the LW group. Amino acids and lactate are important precursors of glucose in newborn calves [5]; therefore, calves in the HW group had greater availability of substrates to synthesize energy reserves. The increase in 3-hydroxybutyrate and glucose in the blood plasma of the LW group may come from lipolysis caused by stress from negative energy balance [6].

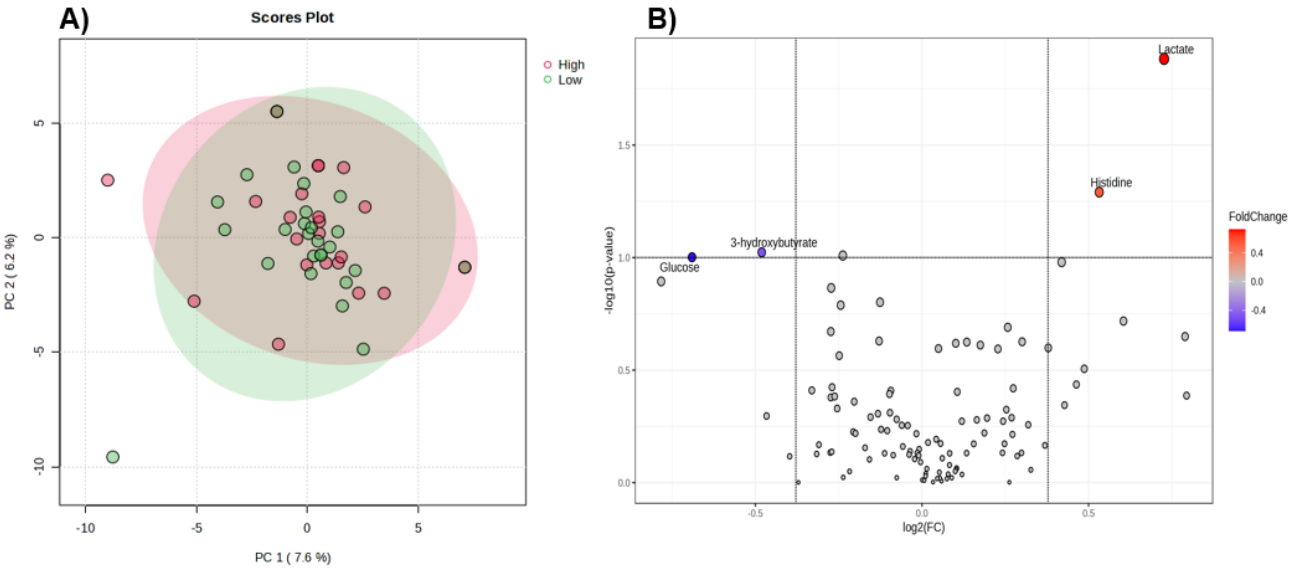

Figure 1. A) Principal component of analysis from calves with high and low birth weight; B) Volcano plot of metabolites from calves with high and low birth weight

CONCLUSION

Nellore calves with greater birth weight may presented a neoglicogenic metabolism by lactate and aminoacids, and the metabolism of lower birth weight calves appears to be correlated with lipolytic metabolic pathways to generate energy.

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EFFECT OF SEX AND AGE ON BEEF TENDERNESS IN PASTURE-RAISED CATTLE

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I. INTRODUCTION

The consumption of beef has been increasing over the years and becoming increasingly scrutinized by consumers, who associate their perceptions with the quality of the meat and thus direct their preferences for purchasing decisions [1]. This decision is mainly guided by tenderness, which can be determined through intrinsic muscle characteristics, fat distribution, age, and sex of the animals [2] [3]. Age and sex have long been discussed factors due to being ante mortem characteristics that can contribute to variations in meat quality through the influence they exert on its composition and consequently, its tenderness [4]. The aim of this study was to evaluate the tenderness of meat from castrated male and female cattle of different ages, with British genetics, finished in an extensive system.

II. MATERIALS AND METHODS

Samples of striploin from 95 British genetics cattle carcasses, obtained from a Uruguayan slaughterhouse, were used for this study. The samples were collected from 31 females and 64 castrated males, with each group composed of animals with 4, 6, and 8 permanent incisor teeth (PIT), with 25 animals having 4 PIT, 26 animals having 6 PIT, and 44 animals having 8 PIT. The carcasses were previously weighed and after collection, the samples were aged for 15 days, frozen, and sent to the laboratory for evaluation of subcutaneous fat thickness, marbling, visually supported by photograpic standards (Quallity Grade- USA) and instrumental tenderness analysis, using the Warner-Bratzler Shear Force method [5]. The obtained data were analyzed by factorial ANOVA, and the means were compared by Tukey's test ($P < 0.05$), using Statistica 10 software (StatSoft, USA, 2010).

III. RESULTS AND DISCUSSION

Regarding age, differences were observed between younger animals (PIT 4 and 6) and older animals (PIT 8), where older animals presented heavier carcasses and higher marbling compared to carcasses of younger animals. However, when observing subcutaneous fat thickness and tenderness, no differences were found among the three age groups evaluated. Regarding sex, no significant differences were observed between castrated males and females when evaluating weight, subcutaneous fat thickness, marbling, and tenderness.However, when relating sex to age, differences were found between castrated males of the three age groups and females of the three age groups regarding carcass weight. It was also found that older castrated males (PIT 8) had marbling in greater quantity compared to the other evaluated groups.

The obtained results show that the data of subcutaneous fat thickness and tenderness in the applied treatments were statistically similar, however, when relating the characteristics of fat distribution and carcass weight to sex and age, differences were observed. With increasing age, the organoleptic characteristics of meat may vary due to changes in collagen with increased cross-linking [6]. However, other factors related to the genetics of the animals may also influence these changes. Regarding sex, it is known that differences in weight and fat cover are justified by the influence that the metabolism of different sexes can promote in the composition of the animals' musculature [7].

Table 1. Means ± standard deviation of weight, subcutaneous fat thickness, marbling, and tenderness of samples from castrated males and females of different ages.

Treatments	n	Weight (kg)	SFT (mm)	Marbling	Tenderness (kg)
Sex					
Castrated Male	64	155,40 ± 1,32 [a]	5,88 ± 0,30 [a]	3,36 ± 0,13 [a]	3,23 ± 0,08 [a]
Female	31	140,98 ± 3,91 [a]	6,87 ± 0,53 [a]	4,00 ± 0,25 [a]	3,52 ± 0,11 [a]
Age					
PIT 4	25	143,88 ± 3,61 [b]	5,48 ± 0,55 [a]	2,84 ± 0,16 [b]	3,26 ± 0,16 [a]
PIT 6	26	148,75 ± 3,73 [b]	6,04 ± 0,39 [a]	3,35 ± 0,23 [b]	3,30 ± 0,11 [a]
PIT 8	44	155,70 ± 1,84 [a]	6,70 ± 0,43 [a]	4,11 ± 0,18 [a]	3,39 ± 0,09 [a]
Sex x age					
CM x 4	21	150,62 ± 1,85 [a]	5,38 ± 0,62 [a]	2,95 ± 0,18 [b]	3,23 ± 0,17 [a]
CM x 6	21	154,79 ± 2,36 [a]	6,43 ± 0,44 [a]	3,43 ± 0,26 [b]	3,28 ± 0,14 [a]
CM x 8	22	160,54 ± 2,19 [a]	5,82 ± 0,50 [a]	3,68 ± 0,22 [ab]	3,19 ± 0,11 [a]
F x 4	4	108,53 ± 5,89 [b]	6,00 ± 1,22 [a]	2,25 ± 0,25 [b]	3,39 ± 0,41 [a]
F x 6	5	108,53 ± 5,89 [b]	6,00 ± 1,22 [a]	2,25 ± 0,25 [b]	3,39 ± 0,41 [a]
F x 8	22	108,53 ± 5,89 [b]	6,00 ± 1,22 [a]	2,25 ± 0,25 [b]	3,39 ± 0,41 [a]

*Subcutaneous Fat Thickness (SFT); Permanent Incisor Teeth (PIT); Castrated Male (CM); Female (F). The same letters in the same column do not differ statistically according to Tukey's mean test (P > 0.05).

IV. CONCLUSION

It was found that fat distribution and weight differed, while subcutaneous fat thickness and tenderness were statistically similar according to the treatments applied. The study of fat thickness, marbling and tenderness shows that despite differences in ante mortem factors, tenderness is not always directly affected. This study may provide more efficient selection of animals that better meet consumer expectations.

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INFLUENCE OF CASTRATION ON MEAT COLOR OF NELLORE CATTLE FED A DIET HIGH OR LOW IN CONCENTRATE THROUGHOUT FATTENING

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I. INTRODUCTION

Castration and diet can considerably influence carcass weight, affect final pH, and meat color [1, 2], and consequently influence consumers' purchase decisions [3]. Therefore, the goal of this study was to evaluate the beef color of Nellore young bulls and steers fed a high or low-concentrate diet throughout fattening.

II. MATERIALS AND METHODS

A total of 28 young Nellore cattle, averaging 295.6 ± 8.05 kg of body weight and 8.0 ± 0.07 months of age, were used in this study. Half of the calves were randomly selected for surgical castration one week after weaning. Post-weaning, calves were confined for rearing and subsequently adapted to two finishing diets with different roughage:concentrate ratios:50:50 and 15:85. Therefore, four experimental groups were obtained: Steers 50:50, N=7; Steers 15:85, N=7; Bulls 50:50, N=7; and Bulls 15:85, N=7. The animals were slaughtered after 122 days of feedlot trial, with an average weight of 454.4 ± 30.0 kg. The pH was measured in the *Longissimus lumborum* (LL) muscle, using a portable pH meter, Mettler Toledo Pro2Go, and a digital food thermometer (-50ºC to +300ºC) used to measure the temperature, 24 hours after slaughter. After 24-hour chilling period, 2.54 cm thick steaks were obtained from the Longissimus muscle between the 12th and 13th ribs for water retention capacity [4], meat and fat color analysis. The color measurements were performed using a Hunter MiniScan EZ colorimeter (4500L; Hunter Associates Laboratory, Inc., Reston, Virginia, USA). All data were analyzed as a completely randomized design following a 2x2 factorial arrangement of treatments (2 sexual conditions and 2 roughage to concentrate ratios). Analysis of variance (ANOVA) was performed to evaluate the effect of main factors and interaction on carcass and meat traits, using the GLM procedure of SAS. Once detected significant effect ($P \leq 0.05$) for sexual condition at diet or interaction, treatments were compared by Tukey's test. Also, tendency was assumed when $0.05 < P \leq 0.10$.

III. RESULTS AND DISCUSSION

The final pH of the bull group tended to be higher than the pH of the steer group ($P = 0.082$). A towards a sexual condition x diet interaction was observed for meat pH ($P = 0.058$). Compared to steers, bulls are more susceptible to pre-slaughter stress, leading to the production of meat with a higher pH and darker cut [5]. The same can happen with animals that have received a less energetic diet, with low muscle glycogen reserves [6]. Which can ultimately affect the profitability of the beef industry [7]. There was a difference in the beef color component b^* for diet ($P = 0.048$) and a trend for sexual condition ($P = 0.068$) indicating a redder color for cattle in the heavy group. On the other hand, fat color was not affected by diet and sexual condition ($P > 0.05$ for component color), but interaction between sexual condition and diet was observed for fat color.

Table 1 – Meat and fat color of Nellore cattle fed a diet high or low in concentrate throughout fattening.

	Steers		Bulls			*P- value*		
	50:50	15:85	50:50	15:85	SEM	Sexual condition	Diet	Sexual condition*Diet
pH 24h	5.41^{A}	5.42^{A}	5.49^{A}	5.49^{A}	0.02	0.082	0.632	0.058
T 24 h, °C	10.93^{A}	11.27^{A}	10.76^{A}	11.80^{A}	0.22	0.310	0.506	0.392
WRC, %	20.02^{A}	22.09^{A}	19.93^{A}	21.90^{A}	0.58	0.961	0.157	0.295
Beef Color								
*L**	41.24^{A}	42.30^{A}	41.09^{A}	42.35^{A}	0.33	0.854	0.126	0.085
*a**	13.54^{A}	13.15^{A}	13.10^{A}	14.34^{A}	0.28	0.303	0.109	0.944
*b**	12.13^{A}	12.16^{A}	11.32^{B}	12.26^{A}	0.21	0.068	0.048	0.108
Fat Color								
*L**	65.64^{A}	67.54^{A}	67.19^{A}	67.68^{A}	0.46	0.340	0.741	0.770
*a**	9.61^{A}	8.68^{A}	8.68^{A}	10.02^{A}	0.33	0.300	0.253	0.993
*b**	19.95^{A}	19.12^{A}	18.93^{AB}	18.41^{B}	0.32	0.209	0.690	0.017

SEM: Standard error of the mean; For each variable, within a row, means without a common superscript letter are significantly different. Significant differences at 5% probability ($P \leqslant 0.05$). Tendency was assumed when $0.05 < P \leq 0.10$.
T: Temperature 24 h (°C); WRC: water retention capacity.

IV. CONCLUSION

This study indicates that the color of meat from carcasses with normal pH may be more influenced by diet than by castration.

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FASTER PRE *RIGOR* PH DECLINE IS ASSOCIATED TO GREATER MYOFIBRILLAR FRAGMENTATION IN *TRICEPS BRACHII* MUSCLE OF EXCITABLE *BOS INDICUS* CATTLE

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I. INTRODUCTION

Beef from cattle with excitable temperament is associated with reduced quality. The stress responsiveness throughout production period can be aggravated before slaughter, when cattle is facing a great number of novelty. Stress can negatively influence Ca^{2+}-dependent proteolysis of skeletal muscle [1], which could be worst in muscles with greater contribution of oxidative fibers. On the other hand, pH and temperature declines can be determinant of protease activation and dependent of cattle stress responsiveness. Therefore, the present study was conducted to verify the relationship between pH and temperature decline in the *Tricpes brachii* (TB) muscle with proteolysis in beef exposed to prolongued aging.

II. MATERIALS AND METHODS

All experimental procedures involving animal care were conducted in accordance with the FZEA/USP Animal Care and Use Committee Guidelines (6493190121). From a group of 72 Nellore males, a sub-group of 23 was selected based on temperament tests. Chute score and flight speed were determined and used to calculate temperament index [2], that was used to classify animals either as excitable or calm. Care was taken to select progenies from several bulls, as well as to represent two genders (non-castrated and immunocastrated). The pH and temperature decline were recorded at 1, 3, 6, 9 and 24h *post mortem* in the TB muscle, which was excised from carcasses at 24h. Small pieces from the TB muscle were vaccum packaged and aged for 1, 7, 14 and 28 days. After each aging period the muscles were frozen using liquid nitrogen and stored to further myofibrillar fragmentation index (MFI) analysis [3]. The statistical model included the gender as a block, the fixed effect of temperament time, and interaction, with random effects of animal and slaughter. For pH and temperature the factorial design used was 2 × 5; 2 temperaments (excitable or calm) and 5 times *postmortem*, while for MFI it was 2 × 4 aging periods. The mixed model was tested using SAS software and time/aging was considered a repeated measure.

III. RESULTS AND DISCUSSION

The pH decline was affected by the interaction between temperament and time *post mortem* (P = 0.02). The pH in TB from calm animals showed greater values at 6 and 9h *post mortem* compared to pH in the TB from excitable cattle (Figure 1A). Addittionally, the pH in TB from calm animals at 3 and 6h post mortem were similar. Temperature decline in the TB was affected (P < 0.001) by time *post mortem* (Figure 1B). The TB fragmentation index was affected by the interaction between temperament and aging period (Temperament × Time: P = 0.04; Figure 2). Fragmentation index in the TB from calm animals was similar at the initial aging period, but improved from 7 to 14 and from 14 to 28d. On the other hand, TB from excitable animals improved from 1 to 7, and from 7 to 28d of aging, with 14d showing intermediate values. At 7d of aging, MFI was greater in TB from excitable animals

when compared to calm animals, which was not expected. However, adrenergic stimulation of calcium pumps, at ATP expenses, may accelerate glycolysis [4]. Therefore, the faster pH decline in TB from excitable cattle positively contributed to early calpain-1 activation, once decreasing ATP concentrations and acidic conditions can lead to sarcoplasmic reticulum disfunction, increasing sarcoplasmic Ca^{2+} [5]. No differences were observed between MFI values at final period, with overall values of 103.6 ± 2.72.

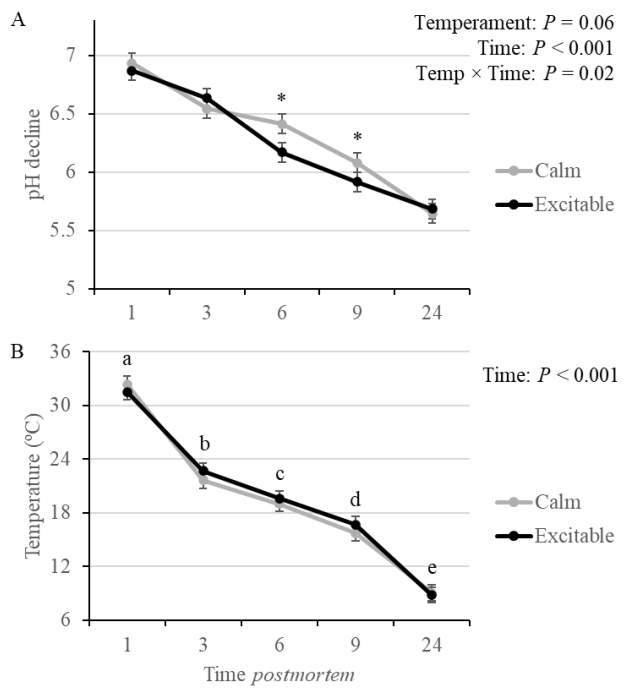

Figure 1. pH (A) and temperature (B) decline *postmortem* in *Triceps brachii* muscle from Nellore male classified as calm or excitable

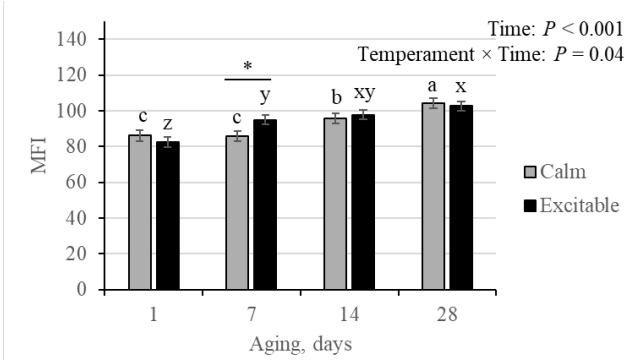

Figure 2. Myofibrillar fragmentation index (MFI) in *Triceps brachii* muscle from Nellore male classified as calm or excitable and aged up to 28 days. *Difference between temperament within aging period. [a,c]Difference during aging within calm temperament. [x,z]Difference during aging within excitable temperament.

IV. CONCLUSION

Myofibrillar fragmentation index in the *Triceps brachii* muscle was influenced by cattle temperament and it is associated with rate of pH decline. Faster pH decline was positively associated with earlier fragmentantion, which was verified in muscle from excitable animals.

ACKNOWLEDGEMENTS

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DETERMINATION OF THE PROPORTION OF PORK AND CHICKEN IN FRESH SAUSAGE BY REAL-TIME PCR

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I. INTRODUCTION

This work aimed to use the real-time PCR (qPCR) technique to quantify the quantity of chicken and porcine simultaneously in samples of fresh chicken sausage, which may contain pork protein in its composition due to the use of pig parts with high fat content, important for contributing to the specific flavor, aroma, and consistency of raw sausages [1]. The analyzed samples served as study material to test the qPCR technique to quantify the DNA of the two species in a meat product since it contains several ingredients, including salt, fat, and sugar, among others, which can make DNA extraction more laborious. The work becomes relevant since the definition of a method that can differentiate and measure the levels of different animal species simultaneously, using the multiplex technique, which makes quantification more agile, can be applied in cases of suspected adulteration in meat products, whether intentional or not.

II. MATERIALS AND METHODS

The samples of fresh chicken sausage were prepared in a pilot plant at the Meat Technology Center of the Institute of Food Technology (CTC-Ital). In sample 125*, the chicken cut used was the chicken breast with skin, and in sample 126*, cuts of chicken thighs and drumsticks with skin. In both samples, pork jowl was added in a smaller quantity. The remainder of the bulk from both samples comprised the basic ingredients for the preparation of fresh sausage.

DNA extraction was carried out using the ReliaPrep™ gDNA Tissue Miniprep System column extraction kit, Promega. The selection of primers used for this study, as well as the hydrolysis probes, was based on the study by Fröder (2022) [2]. Table 1 presents information about the primers and probes used. Two control samples were also tested to confirm the reliability of the results. These samples were prepared in the laboratory with fresh pork and chicken meat, in different proportions (Table 2).

Table 1 – Primers and hydrolysis probes used in this study.

Name	Reference gene	Sequence	Target
Porcine-97bp-F	Beta actin (DQ452569.1)	5′-CGTAGGTGCACAGTAGGTCTGAC-3′	*Sus scrofa domesticus; S. scrofa*
Porcine-97bp-R		5′-GGCCAGACTGGGGACATG-3′	
Porcine-97bp-P		5′-[FAM]-CCAGGTCGGGGAGTC-[NFQ-MGB]-3′	
Chicken-77bp-F	TGF-β3 (AY685072.1)	5′-CAGCTGGCCTGCCGGC-3′	*Gallus gallus domesticus; G. gallus*
Chicken-77bp-R		5′-GCCCAGTGGAATGTGGTATTCA-3′	
Chicken-77bp-P		5′-[FAM]-TGCCACTCCTCTGCACCCAGTGC-[TAMRA]-3′	

The qPCR technique was applied to quantify the two species under study, and the standard curve method was used, which provides the results of the absolute quantification of the samples. To carry out the method, the QuantiStudio™ 3 Real-Time PCR Instrument – Applied Biosystems, Thermo Fisher Scientific, was used as a thermoclycler. The standard curves for chicken and pork species were constructed separately, creating a series of 5 dilutions in a proportion of 1:10 for each.
For amplification of the curve points and samples, GoTaq® Probe qPCR Master Mix, Promega, was used for a 10 µL reaction, with 1 µL of sample for 9 µL of the master mix, primers and hydrolysis probes, and amplification conditions were: initial cycle at 95 °C/2 min; 40 ciclos: 95 °C/15 s e 54 °C/1 min.

III. RESULTS AND DISCUSSION

Table 2 presents the results obtained from qPCR for the samples of fresh chicken sausage with pork jowl and for the two control samples. Comparing the results of the calculated proportion with the initial proportion of the control samples, it is perceived that they are very similar to the prepared proportion, indicating the reliability of the results. When comparing the content of each species of the sausage samples with the initial proportion column (normalized), it is observed in sample 125* that the result was also very similar to what was expected; however, sample 126* presented a slightly greater deviation than expected for the initial content used in the preparation of the meat product. This can be explained by the difference in the cuts used to prepare the sausages, which may present DNA variation throughout the body structure of the animals studied. It can also be assumed that the equipment used in the preparation of the products may have remnants of the product fabricated previously and may have contributed to the increase in the DNA content of the chicken species in this sample.

Table 2 – Results obtained from qPCR for fresh sausage and control samples.

Sample identification	Proportion of species (%)				DNA (ng/reaction)		Calculated proportion of species (%)(3)	
	Initial(1)		Initial normalized(2)					
	Chicken	Porcine	Chicken	Porcine	Chicken	Porcine	Chicken	Porcine
SF50 (control)	50,00	50,00	50,00	50,00	15,997	15,592	50,64	49,36
SF80 (control)	80,00	20,00	80,00	20,00	6,289	23,051	21,43	78,57
125*	73,85	8,00	90,23	9,77	15,397	1,915	88,94	11,06
126*	73,85	8,00	90,23	9,77	17,432	0,915	95,01	4,99

(1) Proportion of mass used in sample preparation. In the case of sausage samples – 125 e 126* - this proportion is related to the total mass prepared, including the rest of the ingredients*

(2) Proportion of the mass used in sample preparation, normalized for sausage samples – 125 e 126* - and excluding the rest of the ingredients, considering only the mass of porcine and chicken protein*

(3) Proportion calculated from the DNA result provided by qPCR

IV. CONCLUSION

The study's findings indicate that the qPCR standard curve technique was found to be effective in quantifying DNA from chicken and porcine species in fresh sausage samples. Additionally, the technique's ability to calculate the proportion of each species was found to be relatively simple, suggesting that it could be a useful tool for identifying fraud in meat products. It is important that the study continues, including the investigation of other species and other meat products.

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CORRELATION BETWEEN LAB VALUES AND MYOGLOBIN FRACTIONS IN DRY-AGED BEEF

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I. INTRODUCTION

The Lab color space is a color model used in digital imaging, representing colors through lightness (*L**) and chromaticity coordinates *a** (redness) and *b** (yellowness) [1]. Unlike other models, *Lab* is designed to be perceptually uniform, meaning changes in values correspond to changes in perceived color. This ensures color measurements align with human visual perception, crucial for assessing meat quality [2]. *Lab* parameters can detect subtle color changes that often correlate with other quality attributes like freshness, tenderness, and juiciness. For instance, the *L** value indicates meat brightness, while a decrease in *a** might suggest a loss of redness linked to freshness [3]. Thus, *Lab* allows standardized color measurements across different meat batches and types, aiding consistent quality control and ensuring products meet color standards required by consumers and regulatory bodies [2]. However, its use in monitoring meat dry-aging processes is rarer [2, 4].
This work evaluates extending the *Lab* model's usefulness for controlling and characterizing beef dry-aging processes. We analyzed the relationship between the mentioned parameters and amounts of various myoglobin fractions, such as purple-red Deoxymyoglobin (DMb) in unoxygenated meat, bright red Oxymyoglobin (OMb) in oxygenated meat, and brown Metmyoglobin (MMb), indicating oxidized meat [5, 6].

II. MATERIALS AND METHODS

Six loins (*L. lumborum*) with the same characteristics were selected, identified as A, B, C, X, Y, and Z, divided into three pieces, and aged for 60 days in a dry aging room. On days 1, 14, 35, and 60, the color *L***a***b** was measured on lean meat at room temperature using a chroma meter (CR410, Konica Minolta Co., Japan) calibrated with a standard white tile. CIE values were measured at three random locations on each sample.
Myoglobin fractions were quantified spectrophotometrically (GENESYS 50 UV-Vis, ThermoScientific, USA) using the method by Krzywicki [7], modified by Suman & Joseph [8], on 1.5 g of each thawed meat sample. After adding 4.0 mL of pH 6.8 phosphate buffer, samples were homogenized for 5 minutes until uniform and then centrifuged at 5,500 RPM for 15 minutes at 4°C. The absorbance of the clarified supernatants was measured at 525 nm, 545 nm, 565 nm, and 572 nm. Krzywicki's equations were used to determine the relative concentrations of DMb, OMb, and MMb based on their characteristic absorbance peaks.

III. RESULTS AND DISCUSSION

As shown in the first two graphs below (Figure 1A and 1B), which separately represent variations in *L**, *a**, and *b** values, and DMb, OMb, and MMb concentrations for sample C, no directly correlatable trend is evident between the CIE values and the levels of different myoglobin forms. Due to the editorial limit of two pages per communication, only the data for sample C is presented. However, the rightmost

graph (Figure 1C) and Table 1 demonstrate a surprisingly accurate correlation between the values of (L^*/40) and the sum of OMb and MMb concentrations, with 83% of the samples showing errors of 15% or less. Table 1 does not present the individual values of all parameters but only the percentage errors, again due to editorial constraints.

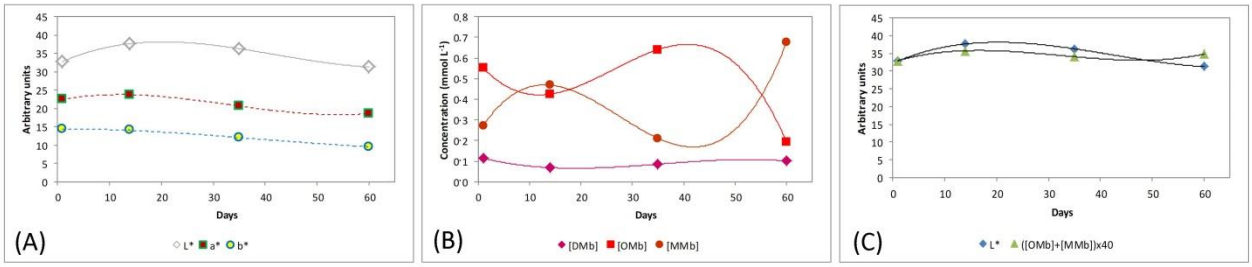

Figure 1. Scatter plots with smooth lines for sample C showing: (A) the variation L^*, a^* and b^* ; (B) [DMb], [OMb] and [MMb]; and (C) the color L^* and the sum of [OMb]+[MMb] with the correction factor of 40, all during the aging days.

Table 1 – Percentage error between measured and predicted Σ[OMb + MMb] based on L^*/40 value

	Aging time (days)			
Samples	1	14	35	60
A	45.26	2.75	-1.13	2.05
B	-4.17	3.71	-9.44	-15.41
C	-17.53	-10.59	-14.80	-12.98
X	-6.03	-19.11	-4.84	-4.66
Y	-10.57	-14.71	4.76	13.78
Z	-9.64	-28.56	-10.94	0.34

IV. CONCLUSION

Despite the relatively low number of samples, the results show a consistent correlation between L^* and the sum of OMb and MMb, representing a small but promising step toward finding and validating new analytical methods for understanding the physical-chemical processes in beef dry aging.

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USE OF ARTIFICIAL INTELLIGENCE TO EVALUATE BEEF CARCASS FAT COVERAGE IN BRAZIL

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I. INTRODUCTION

In meat processing industry's slaughter line, evaluation of the fat coverage on carcasses is carried out by trained technicians, using human vision. The results of this assessment are communicated to industry, indicating proportion of carcasses in different classification standards according to market requirements, being crucial to guide their direction. Slaughterhouses throughout Brazil work to ensure quality of carcasses, especially beef fat coverage, directing products to more than 150 importing countries and to broad domestic market. Classification of carcasses in Brazil continues to be subjective, depending on human evaluation, which, combined with the inherent subjectivity, can lead to partial evaluations that affect redirection of products on market. According to Vadivambal and Jayas [1], estimated accuracy of a subjective visual model ranges from 40% to 50%. Recently, Daniel et al. [2] demonstrated an accuracy of 92.86% using artificial intelligence (AI) in classifying beef carcasses, with a margin of error of 7.14% considered acceptable. The adoption of an autonomous AI-based system for automated classification of beef carcasses can ensure impartiality, accuracy and reliability in data, essential for optimizing targeting of carcasses in accordance with commercial product standards. This will allow generation of fundamental data for decision-making aimed at improving and promoting market standards both nationally and internationally. The objective of this research is to use AI to classify in real time fat coverage of beef carcasses carried out in slaughterhouses, identifying potential in quality and inferring increased industrial efficiency.

II. MATERIALS AND METHODS

The autonomous image capture system installed in slaughterhouse to identify beef carcasses used high-resolution cameras and lenses and built a dataset of 2000 images. Images were captured automatically after animal's slaughter procedure at the exact moment half-carcasses were weighed on scales on overhead rail. These images were recorded, by trained professionals, in three classes according to market product: class 1 – no fat coverage, class 2 – uneven fat coverage, class 3 – standard fat coverage Afterwards, obtained dataset was divided into a training set (1500 images) to train AI models (MOD AI) by ordinal classification [3], and a test set (500 images) to evaluate performance of MOD AI. Based on evaluation results, performance of model trained using reserved test set was evaluated using metrics such as precision, recall, F1-score and confusion matrix to understand model's classification ability (accuracy). And, after MOD AI was validated, it was implemented in a real test environment, a beef carcass slaughterhouse production line, to validate performance and practical feasibility of proposed solution. Performance and feasibility were based on 9,085 images captured and classified by a slaughterhouse technician (on-site classification) compared to classification by AI model.

III. RESULTS AND DISCUSSION

Based on AI model evaluation performance metrics, best model obtained presented 88% accuracy, as shown in Table 1 and Figure 1.

Table 1. Model performance evaluation metrics trained for beef carcass fat coverage classes.

Fat Coverage	Precision	Recall	F1-Score	Support
Class 1	0.92	0.98	0.95	136
Class 2	0.73	0.79	0.76	121
Class 3	0.94	0.86	0.90	243

Table 2. Assessment of fat coverage of beef carcasses when AI model is adopted.

Fat Coverage	*In loco*	MOD AI	If adopted AI	
			Elevate	Decline
Class 1	762 (8.4%)	673 (7.4%)	↑423	---
Class 2	2803 (30.9%)	2418 (26.6%)	↑1349	↓230
Class 3	5520 (60.8%)	5994 (66%)	---	↓1024
Total	9085 (100%)	9085 (100%)	↑1772 (19.5%)	↓1254 (13.8%)

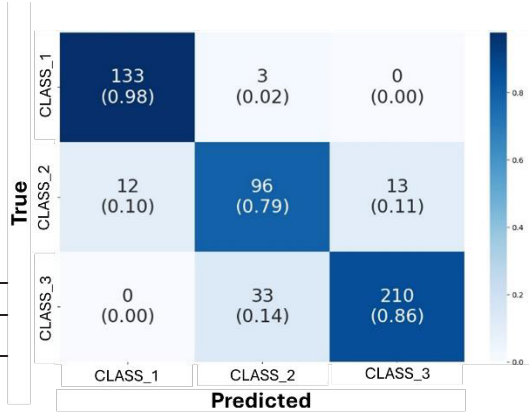

Figure 1. Confusion Matrix Normalized

Application of MOD AI algorithms on 9,085 images of carcasses collected in slaughterhouse showed alignment with local Classifier in 66.7%, and, if applied, in real decision-making in industrial flow (Table 2). However, AI algorithms would classify 3026 carcasses (33.3% of total) differently as follows: AI would consider 1772 (19.5%) carcasses as being of a higher fat coverage class than that assigned by local Classifier, demonstrating a real effective gain in recognition of carcasses with best coverage by a homogeneous and impartial computational system. AI would reposition 1254 (13.8%) carcasses as having a lower fat coverage class than that assigned by local Classifier, which is also a real gain for slaughterhouse's operations, as it more accurately and transparently recognizes conditions of carcasses that are entering industry.

IV. CONCLUSION

Use of AI in slaughterhouse's industrial flow not only provides a homogeneous and impartial evaluation system but can also result in a significant improvement in accuracy of carcass classification. This translates into an optimization of production process, better recognition of quality products and, potentially, greater economic efficiency. It is noteworthy that algorithmic models still need to be validated in an operational environment, requiring continuous development efforts and significant improvements in image capture and synchronization software in real conditions.

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USE OF ARTIFICIAL INTELLIGENCE TO EVALUATE STANDARD OPERATING PROCEDURE (SOP) FOR BOVINE CARCASSES IN BRAZIL

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I. INTRODUCTION

In slaughterhouses, Standard Operating Procedures (SOPs) ensure consistent application of safe practices during processing of beef carcasses, in addition to having a direct impact on profitability of beef carcasses, ensuring a high standard of quality in final products through standardization of cuts, effective removal of unwanted parts and ensuring that carcasses are in ideal conditions for consumption. Among SOPs adopted by slaughterhouses that can influence economy, removal of tallow from pelvis and removal of paw bone are procedures whose inadequate execution directly affects final weight of carcass and, consequently, profitability of slaughterhouse. An effective solution to monitor and ensure correct execution of SOPs is implementation of use of computer vision with artificial intelligence (AI). By implementing AI systems to monitor SOPs, meatpackers can ensure not only product safety and quality, but also operational efficiency and resource maximization, enabling a proactive approach to identifying potential issues. This enables immediate and ongoing corrections to optimize performance and minimize waste, thus promoting better practices and more consistent results in beef processing industry. The objective of this research is to use AI to monitor in real time procedures carried out in slaughterhouses, identifying potential inappropriate practices, resulting in increased industrial efficiency.

II. MATERIALS AND METHODS

The autonomous system for capturing images of beef carcasses installed in slaughterhouse used high-resolution cameras and lenses and built a dataset containing 1500 images. Images were captured automatically after animal's slaughter procedure at exact moment half-carcasses were weighed on scales on overhead rail. Two SOPs were evaluated: pelvic tallow and paw bone. The captured images were annotated by trained professionals, in two classes: class 0 – adequate SOP and class 1 – inappropriate SOP. After annotated dataset, a model trained with YOLOv8 architecture [1] was used, responsible for simultaneous detection and classification, detecting 100% of region of pelvic tallow and paw bone in bovine carcass. For classification of SOPs, it was carried out in a separate model using Resnet101 architecture [2], well consolidated in literature. A training set (80% of captured images) was used to train AI models (MOD AI) and a test set (20% of captured images) was used to evaluate performance of MOD AI. Based on evaluation results, performance of model trained using reserved test set was evaluated using metrics such as precision, recall, F1-score and confusion matrix to understand model's classification ability (accuracy). And, subsequently, final tests were carried out using a separate validation data set to verify robustness and generalization of model, and thus implement it in a real test environment, a beef carcass slaughterhouse production line, to validate performance and practical feasibility of proposed solution. Performance and feasibility were based on 1000 images captured and classified by MOD AI.

III. RESULTS AND DISCUSSION

Based on performance metrics for evaluating AI models, best model obtained presented 80% and 92% for pelvic tallow and paw bone, respectively, as shown in Table 1 and Figures 1 and 2.

Table 1. Performance evaluation metrics of trained model for classes of beef carcass SOPs.

Fat Coverage	Precision	Recall	F1-Score	Support
		Pelvic Tallow		
Class 0	0.80	0.80	0.80	138
Class 1	0.80	0.80	0.80	134
		Paw Bone		
Class 0	0.96	0.93	0.94	258
Class 1	0.95	0.92	0.94	220

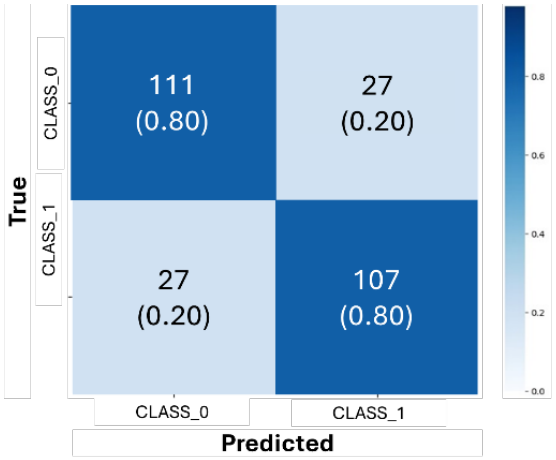

Figure 1. Confusion Matrix Normalized - Pelvic Tallow

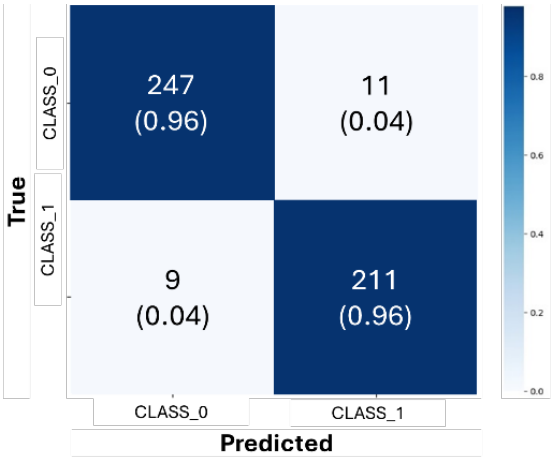

Figure 2. Confusion Matrix Normalized - Paw Bone

Application of Paw Bone verification algorithms, on 1000 images of bovine carcasses, showed 74% of Inadequate SOP. When we consider addition of 210 g (real weighed values) of tarsal bones present in 74% of carcasses, this amounts to an additional disbursement of up to US$ 14,918.40 per month for 18 thousand animals slaughtered. Application of Pelvic Tallow verification algorithms, in 1000 images of carcasses, showed 22% of inadequate SOP, that is, with inadequate removal of tallow from basin. It is estimated that each inadequate SOP adds 150 g (real weighed values) of tallow present in 22% of carcasses, resulting in an additional disbursement of up to US$ 3,168.00 per month for 18 thousand animals slaughtered.

IV. CONCLUSION

With integration of artificial intelligence solutions, slaughterhouses can further improve their processes, achieving higher levels of efficiency and precision in execution of SOPs. It should be noted that algorithmic models still need to be validated in an operational environment, requiring continuous development efforts in real conditions.

ACKNOWLEDGEMENTS

This work was supported by National Council for Scientific and Technological Development (CNPq) (process number: 409694/2022-3).

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MICROBIOLOGICAL QUALITY OF MEAT POULTRY FOODS (VISCERA, NECK AND FEET) AT THE RETAIL LEVEL IN PORTUGAL

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I. INTRODUCTION

One of the most widely consumed meat products across the world is poultry meat, both in developed and developing countries [1]. According to the most recent data, global poultry meat consumption reached 14.88 kg per capita in 2023 [2]. Among poultry meat products, broiler carcasses, cuts, and processed products are the most consumed (~75% of total poultry meat) followed by turkey (~25%) [3]. Poultry meat spoils quickly and even under refrigerated conditions *Enterobacteriaceae*, *Pseudomonas* spp., psychrotrophic and mesophilic bacteria are considered potential spoilers of poultry meat [4]. The contamination of poultry meat by deteriorative and pathogen agents are frequently associated with the slaughtering and processing phases. Typically, 1-day-old broiler chicks are obtained from hatching facilities and transported to grow-out facilities, where they are reared for 4 to 8 weeks before being slaughtered. Carcasses and viscera are subsequently further processed and transported to retail facilities. Once reaching retail, broilers can be sold as whole carcasses, cut parts, or further processed chicken products.

This study was conducted with the main aim of assessing the levels of microbiological contamination (deteriorative) of broiler meat (*Gallus gallus*) products namely viscera (liver, heart, gizzard) and feet and neck purchased from retail markets and supermarkets in package or unpacking conditions.

II. MATERIALS AND METHODS

A total of 72 poultry food products (livers, hearts, necks, gizzards, and feet), purchased in local market and supermarkets in North Portugal were analyzed for selected foodborne and spoilage microorganisms. The spoilage microorganisms analyzed were *Enterobacteriaceae*, *Pseudomonas* spp., moulds and yeasts, total mesophilic and psychrotrophic bacteria. ISO methods were applied and after incubation, typical colonies were counted and results were expressed in log cfu/g. For statistical purposes, when the microorganism count was below the detection limit, it was considered zero log cfu/g.

III. RESULTS AND DISCUSSION

Food products from local market displayed higher counts than from supermarkets for all microorganisms investigated. Regarding *S. aureus*, products from the local market presented on average over 1 log cfu/g (2,34 ± 1,53 vs. 1,01 ± 1,20) than supermarket food, except neck samples that accounted ~ 2 log cfu/g (3,54 ± 1,49 vs. 1,63 ± 1,04) more than supermarkets foods. Higher counts were also presented in non-packaged products.

The microbiological evaluation of spoilage microorganisms in the 72 food products purchased at the retail level in Portugal showed that total aerobes at 7ºC and 30ºC, *Enterobacteriacea*, Pseudomonas spp., and yeasts were present in all samples. At least one mould genus was isolated from 30.6% of the samples. Moulds were more prevalent in the neck (33.3%) followed by the liver (28.6%), feet (37.5%), gizzard (35.7%), and heart (18.8%) samples. In total, five different genera of moulds namely,

Acremonium spp., *Cladosporium* spp., *Verticillium* spp., *Fusarium* spp., and *Penicillium* spp. were identified.

Microbiological counts (mean±sd) of different broilers meat products namely, feet, gizzard, heart, liver and neck are presented in table 1.

Table 1. Microbiological counts (mean±sd) of different broiler products (feet, gizzard, heart, liver and neck).

Sample (N)	Total (72)	Feet (16)	Gizzard (14)	Heart (16)	Liver (14)	Neck (12)	*p*
Moulds and yeasts	0.38±0.65	0.49±0.70	0.48±0.797	0.13±0.342	0.43±0.745	0.38±0.57	NS
Enterobacteriacea	4.39±0.10	4.24±1.02	4.40±1.26	4.64±1.10	4.13±0.779	4.56±0.69	NS
Psychrotrophic	6.03±1.21	6.94±0.913[a]	5.73±1.54[b]	5.79±1.20[b]	5.59±0.864[b]	6.01±0.99[b]	P<0.01
Mesophilic	6.12±1.01	6.93±0.79[a]	5.55±1.23[b]	6.05±0.10[a]	5.77±0.66[b]	6.20±0.77[a]	P<0.001
Pseudomonas spp	4.07±1.17	4.61±0.79[a]	3.74±1.01[a]	4.00±0.82[a]	3.64±0.32[b]	4.14±1.03[a]	P<0.001
E. coli	2.36±1.29	1.78±1.28[a]	1.24±1.08[ab]	2.52±1.02[acd]	2.83±0.85[ac]	3.68±0.77[d]	P<0.001

NS: not significant

Mean counts of total mesophilic and total psychrotrophic were ~6 log cfu/g. Enterobacteriaceae, moulds and yeasts counts were similar in all broiler products with mean counts of 4.39 log cfu/g and 3.15 log cfu/g, respectively. Regarding the total mesophilic counts, significant differences (P<0.001) were observed with the liver presenting lower mean counts of 5.77 log cfu/g and feet the higher counts (6.93 log cfu/g). For psychrotrophic counts, feet presented the highest counts (P<0.001). In case of. Concerning E. coli higher counts were observed in the neck, with values of 3.68±0.77 log cfu/g.

The contamination of poultry meat by deteriorative and pathogen agents is frequently associated with the slaughtering and processing phases [5]. Contamination can occur during processing and contact with the facility's equipment (e.g., grinders, belts, and saws), contact with food handlers, and exposure to other environmental sources. During transport, broilers that are transported in the lower cages are soiled with fecal matter and during slaughter, there is no possibility of removing or washing them before sunburn, which, although its water is being removed, does not prevent the contamination of birds' subject to slaughter on the same day.

IV. CONCLUSION

Rearing on-farms, transport to slaughter, slaughter process, and at the retail market are all stages of food production with implications in hygiene and quality, where contamination can occur. The potential routes of microbial contamination of products throughout the poultry production and supply chain is a challenging in the food industry worldwide.

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Effects of medium voltage electrical stimulation on color during ageing and frozen storage of Nellore beef

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I. INTRODUCTION

A large part of the Brazilian herd comes from the Nellore breed and its crosses. Electrical stimulation has been extensively used in the beef and lamb industry [1]. Electrical stimulation brings rapid benefits to the development of qualitative carcass characteristics such as bright red muscle color faster than in non-stimulated carcasses [2]. This study aimed to evaluate the influence of medium voltage electrical stimulation at three different intensities, 200V (Treatment 200V, T200), 300V (Treatment 300V, T300) and 400V (Treatment 400V, T400) on the color of *post-mortem* beef muscle of *M. longissimus thoracis* - Nellore, both during ageing and during frozen storage

II. MATERIALS AND METHODS

Forty-three Nellore male animals (not castrated) with an average age close to 18 months were selected. After the carcass division, the left sides were submitted to different medium voltage electrical stimulation (ES) of (Treatment 200V, T200), (Treatment 300V, T300), and (Treatment 400V, T400). The right side did not undergo any treatment (control - CON). After 48 h, the carcasses were deboned and 30 cm cuts of *M. longissimus thoracis* were collected, between the 5th and 12th vertebra. One batch (86 samples) was intended for the study of ageing process (14 days/0±2ºC) and the other batch (86 samples) was for the study of frozen storage (180 days/-18±2ºC). A ColorFlex 45/0 spectrophotometer (Hunterlab, Reston, USA) was used. The configurations were: the illuminant was D65, the observer angle was 10° and the aperture for reading the sample was 0.75 cm. The reading was taken using Universal Software version 4.10. and the CIELAB color specification system with the parameters: L* (lightness), a* (red color intensity) and b* (yellow color intensity). The color saturation or intensity index (C^*), the hue or chromaticity angle (h^*) and the total color difference (ΔE^*) were calculated according to Hunter and Harold (1987) [3]. For the statistical analysis of the results of the effect of ES as a main factor, and storage time as a secondary factor, on color parameters were subjected to analysis of variance using the general linear model (Statistica, version 7.0). Voltage and storage time were considered fixed factors and the interaction between them was also evaluated. The data were submitted to analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

For the color parameters L^*, a^*, b^*, C^* and h^*, T200 suggested the highest results compared to the control and all other treatments during ageing time. Regarding ageing time, an increase in L^* values was observed after 7 days and remained constant at 10 and 14 days. For parameters a^*, b^*, C^* and h^* the values increased until 10 days, where they reached their highest values and remained constant at a time of 14 days (Table 1).

For the color parameters a^*, b^**and* C^*, T200 suggested the highest results compared to the control during frozen storage. Regarding frozen storage, a decrease in L^*, a^* and C^* values was observed, showed loss of color. For parameters b^* increased value until 60 days, where they reached their highest values and decreased at 120 and 180 days. h^* values increased until 120 days and remained constant at 180 days (Table 2). All treatments showed satisfactory values for L^* and a^* and higher for b^* [5].

Table 1. Effect of aging process on beef color parameters.

		Voltage (V)				Time (Days)				SEM	*P value*		
											Voltage	Time	Voltage*Time
		CON	T200	T300	T400	3	7	10	14				
Color	*L**	42.89^{AB}	43.71^{A}	42.23^{B}	42.57^{AB}	41.65^{b}	43.11^{a}	43.51^{a}	43.21^{a}	0.134	0.006	<0.001	0.991
	*a**	22.72^{B}	23.60^{A}	22.34^{B}	22.90^{AB}	20.91^{c}	23.21^{b}	23.95^{a}	23.25^{ab}	0.119	0.002	<0.001	0.838
	*b**	15.77^{B}	16.51^{A}	15.16^{C}	15.73^{AB}	13.64^{c}	15.98^{b}	17.09^{a}	16.44^{ab}	0.118	0.001	<0.001	0.674
Chroma (C)*		27.67^{B}	28.81^{A}	27.01^{B}	27.79^{AB}	24.97^{c}	28.19^{b}	29.43^{a}	28.49^{ab}	0.162	0.001	<0.001	0.765
Hue (h)*		34.65^{A}	34.90^{A}	34.04^{B}	34.31^{AB}	33.06^{c}	34.46^{b}	35.41^{a}	35.21^{a}	0.093	0.004	<0.001	0.821
*ΔE**		-	2.42	2.26	2.49	2.43	2.46	2.36	2.30	0.105	0.663	0.953	0.832

* Capital letters superscripted within the same row refer to the significance of the Voltage variation (P<0.05).
* Lowercase letters superscripted within the same row refer to the significance of the Time variation (P<0.05).
* CON: means untreated or control carcasses (n = 43). T200: means electric stimulation at 200 V (n = 15). T300: means electric stimulation at 300 V (n = 15). T400: means electric stimulation at 400 V (n = 13).
* *Hue**: degrees. *ΔE**: total color difference.

Table 2. Effect of frozen storage on beef color parameters.

		Voltage (V)				Time (Days)				SEM	*P value*		
											Voltage	Time	Voltage*Time
		CON	T200	T300	T400	3	60	120	180				
Color	*L**	36,90	37,58	36,79	36,99	41,65^{a}	33,27^{c}	36,53^{b}	36,60^{b}	0,204	0,210	<0.001	0,998
	*a**	16,30^{B}	16,84^{A}	16,45^{AB}	16,61^{AB}	20,91^{a}	16,85^{b}	14,32^{c}	13,77^{d}	0,168	0,042	<0.001	0,950
	*b**	13,33^{B}	13,86^{A}	13,24^{B}	13,57^{AB}	13,64^{b}	14,27^{a}	13,25^{b}	12,71^{c}	0,074	0,028	<0.001	0,948
Chroma (C)*		21,16^{B}	21,89^{A}	21,17^{AB}	21,52^{AB}	24,97^{a}	22,10^{b}	19,54^{c}	18,76^{d}	0,158	0,016	<0.001	0,937
Hue (h)*		39,76	39,93	39,21	39,70	33,06^{c}	40,29^{b}	42,69^{a}	42,70^{a}	0,245	0,323	<0.001	0,952
*ΔE**		-	2,76^{A}	2,14^{B}	2,65^{AB}	2,43	2,63	2,42	2,55	0,109	0,041	0,867	0,841

* Capital letters superscripted within the same row refer to the significance of the Voltage variation (P<0.05).
* Lowercase letters superscripted within the same row refer to the significance of the Time variation (P<0.05).
* CON: means untreated or control carcasses (n = 43). T200: means electric stimulation at 200 V (n = 15). T300: means electric stimulation at 300 V (n = 15). T400: means electric stimulation at 400 V (n = 13).
* *Hue**: degrees. *ΔE**: total color difference.

IV. CONCLUSION

In frozen storage, color parameters remained within acceptable standards. Notably, electrical stimulation at 200 V proved effective in increase color meat in Nellore beef (M. *longissimus thoracis*). The total difference in color between meat treated with ES and untreated meat, both those subjected to ageing process and those frozen storage, were classified as “noticeable” [4], demonstrating that electrical stimulation improves the color of the Nellore beef.

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DYNAMICS OF METABOLITES AND FREE FATTY ACIDS IN BEEF DURING COLD STORAGE

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I. INTRODUCTION

Wagyu beef is characterized by excellent marbling from intramuscular fat and a sweet aroma produced when cooked [1]. In addition, the lipids in Wagyu beef contain high levels of oleic acid, a monounsaturated fatty acid that prevents lipotoxicity [2]. Wagyu beef, rich in oleic acid, is attracting attention as a healthier option for beef production. Metabolomics analysis can provide a comprehensive analysis of metabolite profiles using mass spectrometry. The development of this technique has greatly advanced our understanding of metabolites closely related to meat aroma, texture, marbling, and color [3]. This study uses omics analysis, including metabolomics, to examine changes in metabolites and lipids in beef during cold storage and propose an optimal aging period.

II. MATERIALS AND METHODS

1. Beef Samples and Wet Aging Conditions

For our study, we obtained commercially available loin (Longissimus thoracis) and Round (adductor muscle) from Japanese Black Wagyu, Holstein-Friesian, and crossbred Wagyu (F1; Japanese Black and Holstein-Friesian) cattle. These samples were then subjected to cold storage, with the loin and round muscles divided into 5 equal portions and vacuum-packed in oxygen barrier film (Varialon S, Asahi Kasei Corporation, Tokyo, Japan). The samples were frozen immediately after packaging on Day 0, and the remaining portions were stored in a refrigerator for 10, 20, 30, and 40 days. We divided the right side for omics analysis and the left for sensory evaluation and analysis of various nutritional components. Our findings from this meticulous process will contribute significantly to the understanding of beef quality control during cold storage.

2. Metabolomics Analysis

Metabolomics analysis for various amino acids and organic acids in beef was performed by GC/MS analysis using Shimadzu GCMS-QP2010 Ultra and a DB-5 capillary column. For sample processing, frozen beef samples (1 g) were ground, and homogenates were centrifuged with a solvent containing sinapic acid. The supernatant was vacuum-dried, derivatized, and subjected to GC/MS.
The peaks obtained were analyzed using GC/MS Metabolite Database v.2 [4].

For lipid analysis, Lipids were extracted from a 1 mg Bligh and Dyer variant sample using nitrogen gas drying and methanol redissolution, followed by LC-MS/MS analysis. The analysis was performed on a DIONEX UtiMate 3000 system with an L-column3 C18 column and Q Exactive Plus detector. HPLC utilized solvents with ammonium formate and ammonium hydroxide, maintaining a column temperature of 40°C, 10 µL injection volume, and 0.1 mL/min flow rate. MS settings included Full MS/dd-MS2 mode, scanning from 200 to 1800 m/z. Various internal standards were used for different lipid classes. TG molecular species were identified using Lipid Search software [5].

3. Free Fatty Acids analysis

Total lipids from 5 g of ground beef were extracted using methanol: chloroform (1:1) with heneicosanoic acid as an internal standard. FFAs were isolated, methyl esterified, and dehydrated with sodium sulfate. Samples were analyzed by GC-FID using a Shimadzu GC-2010 plus with an SP-2560 column [6].

III. RESULTS AND DISCUSSION

This study examined the effects of wet aging on several types of beef (Japanese Black Wagyu, Holstein-Friesian, and crossbred Wagyu) during cold storage. Loin and Round were stored in a refrigerator for up to 40 days. Sensory evaluation showed that wet aging enhanced umami, richness, and continuity of taste over time, with minimal changes in saltiness, sweetness, and acidity. GC/MS metabolomics analysis identified significant changes in metabolites such as amino acids, organic acids, and nucleotides. Principal component analysis classified metabolites into early, middle, and late stages of storage, and a heat map highlighted temporal changes in metabolite concentrations. Amino acids like leucine, tyrosine, and citric acid cycle metabolites increased, while creatinine and glucosamine 6-phosphate decreased. Lipid analysis revealed stable overall lipid composition but significant increases in free fatty acids during cold storage. These findings suggest that wet aging alters the taste of beef through changes in taste-related metabolites and lipid composition, enhancing flavor and quality.

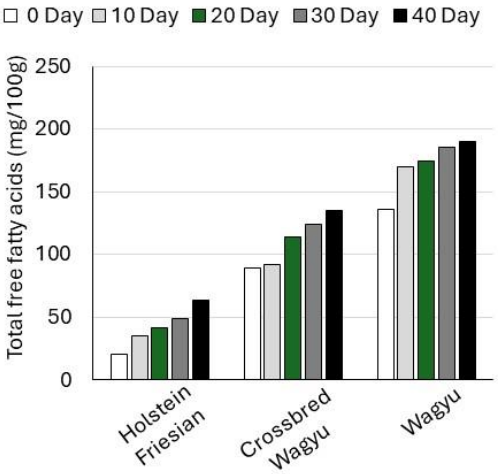

Figure 1. Increase in free fatty acids due to cold storage

IV. CONCLUSION

This study utilized metabolomics analysis to investigate the effects of wet aging on several beefs during cold storage. Initially, sensory evaluation confirmed that wet aging enhanced the umami and kokumi taste of hybrid Wagyu beef. GC/MS-based metabolomics analysis revealed significant changes in the levels of key taste-related metabolites, including glutamate, tryptophan, phenylalanine, acetyl-lysine, xylulose, citric acid, hypoxanthine, and creatinine. Additionally, lipid analysis indicated a significant increase in linoleic acids, which are precursors to aromatic compounds, due to wet aging. This study suggests that the changes in the taste of Wagyu beef resulting from wet aging are linked to alterations in taste-forming elements, including free amino acids, organic acids, and free fatty acids.

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METABOLOMIC PROFILE OF MEAT FROM DIFFERENT ZEBU BREEDS

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I. INTRODUCTION

The Brazilian meat production industry relies heavily on *Bos taurus indicus* (zebu) animals due to their notable rusticity and parasite resistance. A subset of this production is derived from purebred animals, which have been documented to possess specific genetic lineages. It is of paramount importance to conduct research into the metabolite compounds present in different cattle breeds, as these compounds significantly influence meat quality, including aspects such as flavor, tenderness, and nutritional value. The metabolomic profiles of these animals can be understood to lead to the standardization of meat quality, increased commercial value, and the selection of desirable traits for genetic improvement. The objective of this study was to compare the metabolomic profiles of meat from various Zebu breeds, thereby emphasizing the significance of such analyses in improving meat quality.

II. MATERIALS AND METHODS

A total of 110 purebred uncastrated young bulls from four zebu breeds were kept under the same conditions since weaning: Brahman (n = 17), Guzerá (n = 25), Sindi (n = 23) and Tabapuã (n = 41). The animals were fed on pasture for 10 months with balanced dietary supplementation, and finished for 120 days. Following the slaughter of the animals, meat samples (*Longissimus lumborum*) were collected and their metabolomic profiles were analyzed in accordance with the methodology described by Matias et al. [1]. The identification and quantification of metabolites were carried out through the analysis of spectra using the Chenomx NMR Suite Professional software (Chenomx INC., Edmonton, Canada). The data analysis was performed using MetaboAnalyst (Version 6.0, https://www.metaboanalyst.ca/MetaboAnalyst/) employing principal component analysis (PCA) and fold change (Figure 1).

III. RESULTS AND DISCUSSION

In the PCA, it was possible to observe an overlap between the cattle breeds, indicating similarity in the meat metabolomic profile of the different Zebu breeds analyzed. However, a more detailed analysis through fold change revealed some metabolites differences between groups. Carnosine was highest in Tabapuã, followed by Sindi, Guzerá, and Brahman. Malonate was highest in Sindi, followed by Guzerá, Brahman, and Tabapuã. Anserine was highest in Sindi, followed by Tabapuã, Guzerá, and Brahman. Variations in meat metabolism can lead to differences in meat properties. For instance, carnosine and anserine, dipeptides associated with antioxidant capabilities, can impact postmortem meat quality and preventing lipid peroxidation. Both metabolites exhibited similar patterns across breeds, with higher concentrations found in Sindi and Tabapuã cattle, potentially influencing meat postmortem and quality traits. These disparities may stem from the evolutionary and selective processes unique to each breed. Ramos et al. [2] elucidated those adaptations in *Bos taurus indicus* influence muscle physiology, enhancing cell survival and resilience to stress, thereby safeguarding cells from premature death and delaying tenderization.

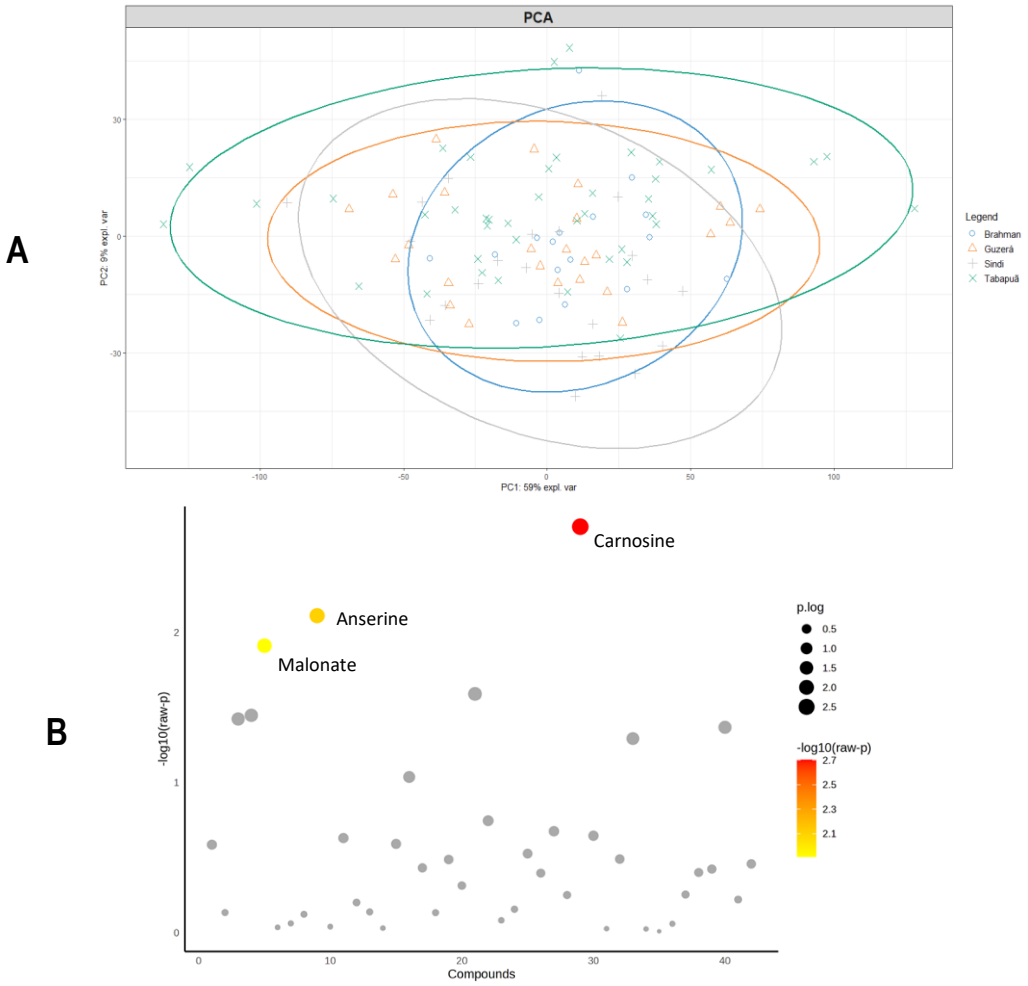

Figure 1. Principal component of analysis (A) and Fold Change analysis of meat metabolic profile between Zebu breeds (B).

IV. CONCLUSION

Although Zebu breeds appear to have a degree of similarity in their meat metabolomic profiles, changes in the levels of anserine and carnosine may suggest differences in postmortem metabolism and potentially great antioxidant capacity in Sindi and Brahman cattle.

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EFFECT OF A RECYCLABLE FILM ON THE COLOR AND SENSORY EVALUATION OF FROZEN 'BLACK PIG' MEAT

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I. INTRODUCTION

'Black pig' (AlentejanoxDuroc) meat products, valued for their sensory qualities and high nutritional value[1], are popular in southern Europe[2]. These crossbreeds produce meat rich in oleic acid and lower in saturated fats. With environmental concerns rising, biodegradable and recyclable packaging is being explored[3,4]. This study found that recyclable packaging effectively maintains the quality of frozen 'secretos' from crossbred pigs stored for 24 months at -18°C, offering an eco-friendly alternative to conventional plastics.

II. MATERIALS AND METHODS

Sampling and sample preparation: 'Secretos' meat samples from the *latissimus dorsi* muscle were prepared from three production batches for each packaging type: recyclable and conventional. The meat was cryogenically frozen at -80ºC for 10 minutes to a final temperature of -18ºC, then vacuum-packed with three 150g pieces per bag and stored at -18ºC with 100% relative humidity for 0, 6, 12, 18, and 24 months.

Colour: Color measurements were conducted using the CIELab system using a Konica Minolta colorimeter (CR 400) with D65 illuminant. Five readings were taken at different locations on each raw sample, both on meat and fat.

Sensory analysis: The sensory analysis was conducted by a panel of 10 trained tasters using a quantitative descriptive analysis with a 0-100 scale. The panel was trained according to ISO 8586-1:1993 [5] and tests were conducted in a sensory analysis room per ISO 8589:2012 [6]. The meat was cooked to a core temperature of 75°C before evaluation.

Statistical analysis: The data was processed using Statistica 12. A multifactorial ANOVA was conducted, considering packaging type (conventional and recyclable film) and shelf life (0, 6, 12, 18, and 24 months). Means were compared using Tukey's HSD test.

III. RESULTS AND DISCUSSION

Color analysis results (table 1) showed no significant differences in a* values between recyclable film and control samples in both meat and fat throughout the trial.

Table 1 – Colour measurement (L*, a*, b*) in meat (1) and fat (2) of 'black pig' *secretos*. Values presented as mean ± standard deviation. In the same column, different letters represent significant different means (p<0.05)

1)

		Meat		
Time (month)	Modality	L*	a*	b*
0	Control	58,5a±7,6	11,7a±1,8	42,7a±7,9
6	Control	74,5b±10,4	7,3a±4,3	55,0b±10,2
	Recyclable Film	76,6b±7,8	8,2a±3,5	62,8b±11,4
12	Control	68,9b±7,2	10,4a±2,5	55,9b±6,4
	Recyclable Film	69,2b±10,8	8,9a±2,9	54,6b±8,8
18	Control	71,4b±1,4	9,7a±0,6	56,1b±2,2
	Recyclable Film	71,2b±0,8	9,7a±0,6	55,9b±1,5
24	Control	71,3b±0,7	9,7a±0,5	56,0b±1,1
	Recyclable Film	71,3b±0,7	9,7a±0,5	56,0b±1,2

2)

		Fat		
Time (month)	Modality	L*	a*	b*
0	Control	75,9a±3,3	5,9a±1,0	57,6a±8,0
6	Control	76,7a±7,1	7,7a±3,9	57,6a±8,8
	Recyclable Film	78,9a±5,2	7,6a±2,9	60,5a±8,2
12	Control	66,2a±10,4	9,4a±2,9	48,2a±11,7
	Recyclable Film	72,2a±9,3	9,5a±3,1	56,6a±6,9
18	Control	75,2a±1,9	9,2a±0,8	56,3a±2,3
	Recyclable Film	75,5a±1,8	9,2a±0,6	56,3a±2,1
24	Control	75,3a±1,4	9,2a±0,5	56,3a±1,9
	Recyclable Film	75,3a±1,4	9,2a±0,5	56,3a±1,9

Hawthorne's study on pork meat [7] found significant b* fluctuations with modified atmosphere packaging, comparing conventional and recyclable films.

The sensory evaluation showed no significant differences in color intensity ($p>0.05$) or fibrosity ($p>0.05$) between packaging methods over time. Control samples were significantly harder ($p<0.05$), juicier($p<0.05$), and had a higher flavor intensity($p<0.05$). No off-colors or unwanted flavors were detected, and tasters could not distinguish between packaging methods over 24 months of frozen storage. Studies by other authors found no differences in sensory attributes when comparing the use of modified atmosphere for preserving pork meat with conventional and recyclable film [7,8]. Packaging type can affect meat sensory perception [7–9]. Hawthorne [7] found no significant differences in sensory attributes between conventional and recyclable films using modified atmosphere. Several authors reported that vacuum packaging positively impacts sensory parameters, particularly those related to oxygen presence, such as color [10,11].

Table 2 – Effect of film type on sensory evaluation of 'black pig' *secretos*. Values presented as mean ± standard deviation. In the same column, different letters represent significant different means ($p<0.05$).

Time (month)	Modality	Colour Intensity	Off Colours	Hardness	Fibrosity	Juiciness	Flavour Intensity	Negative Flavours	Overall Appreciation
0	Control	58,0±18,2	0±0	50,8abc±4,1	21,0±24,0	70,7b±11,1	72,6abc±11,0	0,3±1,8	73,6±11,1
6	Control	61,2±21,8	0,6±2,4	51,78abc±7,1	18,7±23,1	69,4ab±13,0	74,4abc±11,9	0±0	74,8±10,8
	Recyclable Film	61,8±14,7	0,6±2,7	49,7abc±10,3	14,2±15,2	66,6ab±10,9	70,2abc±11,2	0±0	72,4±10,9
12	Control	60,7±9,8	0,5±1,0	51,8a±3,2	18,7±16,6	69,2ab±5,2	74,0b±6,8	0±0	74,5±5,6
	Recyclable Film	61,3±8,2	0,5±01,0	49,8abc±1,9	14,2±9,3	66,5ab±6,2	69,7ac±6,4	0±0	72,1±5,9
18	Control	59,8±5,1	0,5±0,8	51,7ac±2,3	18,5±13,4	68,8ab±2,6	73,5bc±5,2	0±0	73,9±3,6
	Recyclable Film	60,5±4,1	0,5±0,8	49,7bc±0,8	13,9±7,3	65,8a±3,5	69,1a±4,6	0±0	71,6±3,4
24	Control	59,9±3,1	0,5±0,7	51,8a±1,9	18,2±11,6	68,7ab±1,6	73,7b±4,2	0±0	74,0±2,7
	Recyclable Film	60,5±2,3	0,5±0,7	49,6b±0,6	13,8±6,3	65,8a±2,4	69,2a±3,7	0±0	71,4±2,5

IV. CONCLUSION

Recyclable films performed similarly to the control in color and sensory evaluation. Despite higher hardness, juiciness, and flavor intensity in the control, there were no significant differences in brightness, color intensity, off colors, fibrosity, negative flavors, or overall appreciation. Tasters could not distinguish between the different packaging methods, making recyclable films a viable alternative for maintaining meat and fat quality over time.

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MEDIUM VOLTAGE ELECTRICAL STIMULATION COMBINED WITH MULTI-FREQUENCY WAVEFORM AND BEEF QUALITY FROM *BOS INDICUS*

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I. INTRODUCTION

Electrical stimulation (ES) is an important tool to improve meat quality; multifrequency waves were developed with a view to having a better effect on the carcass when subjected to the ES process. Acceleration of glycolysis rate, anticipation of *rigor mortis* onset and *post mortem* proteolysis [1] can have a positive effect in beef quality of cattle with resistant pH decline [2]. However, since carcass pH and temperature decline must be well coordinated, it is important to finely regulate the stimulation to achieve the best outcome for tropical adapted cattle. Since previous work was conducted testing medium voltage ES with single wave [3], the aim of present work was to test an alternative protocol for medium voltage ES combined with multi-frequency waveform and its impact on quality of prolonged aged beef.

II. MATERIALS AND METHODS

Carcasses (n=42) from Nellore non-castrated males were randomly selected to be used in the present study. After carcasses were split, the right halves were assigned to one of the following treatments: CON – non-stimulated carcass; SFW – single frequency electric stimulation 300V AC, 15 Hz (single square wave); MFW= 300V AC, 15 and 200 Hz simultaneously (multi-frequency waveform). For both ES treatments a cycle of 4 seconds on and 2 seconds off, with 60 seconds total time application were used. The left carcasses were stimulated within 20 minutes after slaughter while the right carcasses were kept as control. Hot carcasses were weighed (HCW) and chilled for 24h (0 – 2°C). The pH and temperature declines were recorded immediately after ES in both halves, and at 3, 6, 9 and 24h *post mortem*. After chilling, carcasses were ribbed between 12th and 13th ribs for *Longissimus* muscle area (LMA) and subcutaneous fat thickness (SFT) measurements. Following, 4 steaks (2.5 cm tick) of *Longissimus* muscle were taken from each carcass side, vacuum packaged and aged for 1, 7, 14 and 21d. The steaks were used for cooking loss (CL) and Warner-Bratzler shear force (WBSF) determinations. The data was analyzed as a completely randomized design with 14 replications per treatment. The HCW, LMA and SFT were analyzed considering the fixed effect of treatment (CON, SFW, and MFW) and the random effect of slaughter. The pH and temperature decline were analyzed as repeated measurements, considering the fixed effects of treatment, time of measurement and their interaction and the random effect of slaughter (n=3). Carcass nested within treatment was used as the subject of repeated measurement. All analyzes were carried out using the Mixed procedure of SAS.

III. RESULTS AND DISCUSSION

There was no difference in HCW, LMA or SFT among treatments. The average HCW was 316 ± 7.4 kg, LMA was 77.5 ± 1.94 cm^2 and SFT 3.5 ± 0.41 mm. There was a treatment × time interaction for pH decline ($P < 0.001$), with both ES protocols showing lower pH when compared to control in all time

points ($P < 0.05$; Figure 1). When comparing the ES protocols there was a difference only at 3h, when MFW showed greater pH than SFW ($P < 0.05$). No treatment × time interaction was observed for temperature decline, which was also similar among treatments. Temperature decline was affected ($P < 0.001$) by time *post mortem*, as expected. The initial and final average temperatures were 40.5 and 4.4 ± 0.34 °C, respectively, for ES treated carcasses and from 39.9 and 4.6 ± 0.30 °C for control carcasses. A treatment × time interaction was found for CL ($P < 0.001$) with greatest losses observed in steaks from MFW protocol aged for 7d and lowest losses in steaks from SFW protocol aged 1d (37 and 31 ± 0.8 %, respectively). There was a treatment x time interaction for WBSF ($P < 0.001$; Figure 2). The final WBSF values for beef from the ES carcasses were approximately 28% lower than the values for beef from control carcasses.

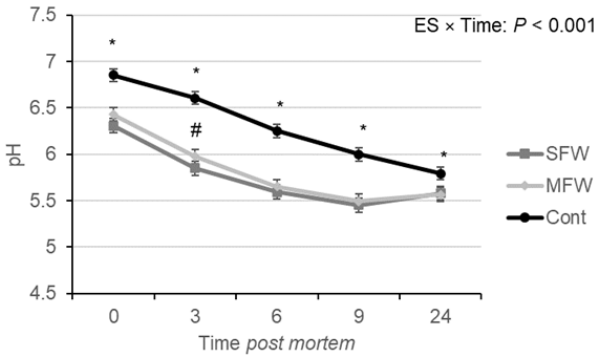

Figure 1. pH decline in *Longissimus* muscle from electrical stimulated (SFW or MFW) and control (Cont) carcasses from non-castrated Nellore males. *Differences between control and ES protocols within aging period ($P < 0.05$). #Differences between ES protocols within *post mortem* period ($P < 0.05$).

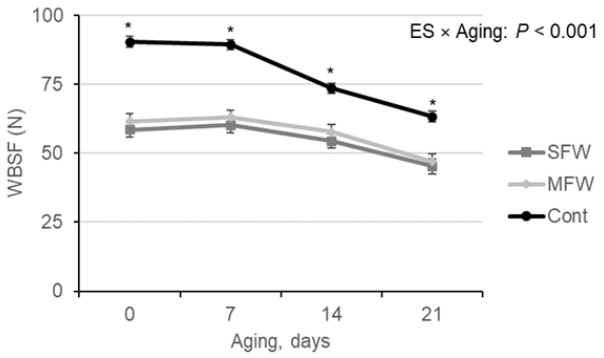

Figure 2. Warner-Bratzler shear force (Newtons) in *Longissimus* muscle from electrical stimulated (SFW or MFW) and control (Cont) carcasses from non-castrated Nellore males. *Differences between treatments within aging period ($P < 0.05$).

IV. CONCLUSION

Both ES protocols were efficient at accelerating the rate of pH decline, with a slightly more paced rhythm for the MFW protocol. The MFW means that several frequencies are applied to the carcass, resulting in a more uniform result regardless of the carcass size, because as multiple frequencies are used, the effect of resonance is mitigated in application. However, the impact on tenderization was similar at the beginning and throughout the aging period for both protocols, that were also considered tender after aging for 21d. Since Nellore is known to have greater calpastatin activity, the adequate pH decline and early calpain activation must be finely adjusted to enhance outcome for industry and consumers.

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Application of tenderness thresholds to Cachena beef

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I. INTRODUCTION

The Cachena is a highly hardy breed of cattle that is part of Portugal's genetic heritage. Breeding these cattle extensively is of great interest in the south-east of the Alentejo, an agricultural region with difficult climatic conditions and poor soils in Portugal. Cachena's animals are small, and the meat is known for its excellent characteristics of texture and flavor [1]. Therefore, the determination of the ideal tenderness is of extreme importance for the producers and consumers. According to several studies on fresh meat, consumers are willing to pay a higher price for meat that is guaranteed as tender [2]. A threshold can be defined as a specific point on the sensory stimulus scale where a transition occurs in a series of sensations or judgments [3].

Ricardo-Rodrigues *et al.* [4] have established a beef tenderness thresholds as a proposal for a reliable and rapid screening index for beef samples, using the texture profile analysis (TPA) and Warner Bratzler shear force (WBSF) methods. According to the model proposed by Ricardo-Rodrigues *et al.* [4], a beef cut is tender when WBSF is below 39.60 N and simultaneously hardness from TPA is below 31.89 N. The use of both parameters allows to establishment of a tenderness screening index for beef samples.

This study aims to assess the tenderness of various cuts of meat from young and adult Cachena animals.

II. MATERIALS AND METHODS

The animals were reared on a farm in Barrancos (South-east of Portugal) and were slaughtered at a slaughterhouse (Beja, South of Portugal). Two groups of animals were slaughtered: (A) 32 young animals aged between 9 and 20 months and the analysis of the tenderloin and sirloin cuts; (B) 33 adult animals aged between 5 and 12 years and the analysis of tenderloin, sirloin, knuckle, and silverside cuts.

The instrumental texture evaluation was performed by assessing the hardness obtained in TPA and shear force obtained in WBSF in grilled meat 72 hours after slaughter. The samples were treated, and the parameters were analyzed as described by Ricardo-Rodrigues *et al.* [4].

Data were analyzed according to the analysis of variance (ANOVA) using StatisticaTM v. 12.0, software (StatSoft Inc., 1984–2014). Differences between groups were identified based on Tukey's Honest Significant Difference (Tukey's HSD) test ($p < 0.05$).

III. RESULTS AND DISCUSSION

The texture results (Hardness in TPA and Warner-Bratzler shear force) obtained for different cuts of meat from young and adult animals are shown in Table 1.

Table 1 – Comparison of hardness and shear force in different beef cuts from young and adult Cachena cattle breeds.

Cachena meat	Cut	Hardness (N)	Shear force (N)
Young animals (A)	Tenderloin	10,92 ± 3,21 **a**	24,35 ± 5,28 **a**
	Sirloin	15,70 ± 7,83 **bc**	32,86 ± 8,31 **bc**
Adult animals (B)	Tenderloin	15,63 ± 4,28 **bc**	27,15 ± 5,01 **abc**
	Sirloin	23,28 ± 8,56 **d**	32,84 ± 7,89 **d**
	Knuckle	25,45 ± 9,99 **de**	38,28 ± 7,76 **d**
	Silverside	29,98 ± 13,46 **e**	42,92 ± 13,44 **e**

Data are expressed as means ± SD. In the same column, different letters (a, b, c, d, and e) represent significantly different means ($p < 0.05$).

The tenderloin of adult Cachena animals exhibits hardness and shear force values similar to those of the sirloin of young Cachena animals. Additionally, it was observed that the instrumental parameter of hardness appears to differentiate the cuts better than the shear force.
According to the established tenderness index [4], Cachena meat from both young and adult animals should be considered tender based on its hardness and shear force values, with an average hardness value of less than 31.89 N and a shear force value of less than 39.60 N. Exception to this is the silverside with shear force values higher than those indicated in the texture index and the hardness values very close to the limit, which aligns with the results obtained from the sensory analysis (data not shown).

IV. CONCLUSION

According to the rapid screening index developed by Ricardo-Rodrigues *et al.* [4], the meat from Cachena cattle can be considered tender, whether it is obtained from young or adult animals, and for the loin, sirloin and steak cuts. Silverside is the only cut of meat that cannot be clearly classified as tender, which is why some precaution is recommended when selling it, as well as any gastronomic preparation other than grilling the meat.
Producers who are interested in this objective assessment of the quality/ tenderness of Cachena meat can use the TPA and WB methods, and the threshold value, as a tool to ensure the tenderness of the meat they sell. As a result, tender pieces of meat can be valued economically, based on the results obtained, in a safe and clear and reliable form.

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MEDIUM VOLTAGE ELECTRICAL STIMULATION AND PELVIC SUSPENSION AS TOOLS TO IMPROVE MEAT TENDERNESS IN NELORE CATTLE

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I. INTRODUCTION

Beef plays a critical role in human nutrition, serving as a rich source of high biological value nutrients. However, it must also meet consumer expectations regarding its physical and chemical properties. Tenderness is the most important qualitative attribute of meat, as demonstrated by consumer acceptance tests [1]. Tenderness, however, is a multifactorial characteristic resulting from the interaction of pre- and post-slaughter factors. Electrical stimulation (ES) of carcasses has been reported as a highly efficient tool for accelerating *post mortem* muscle metabolism and can play a crucial role enhancing the production of tender beef in *Bos indicus* cattle. Additionally, various hanging methods have been tested as a means of manipulating tenderness. Carcass suspension by the pelvic bone (*Tenderstretch*) is a low-cost technique that improves meat quality compared to the Achilles tendon method. Therefore, this study was developed to evaluate the isolated or combined effect of medium voltage ES and carcass suspension method on the shear force (SF) of beef fabricated from *Longissimus* and *Biceps femoris* muscles in Nelore cattle.

II. MATERIALS AND METHODS

Twenty-four carcasses from twelve Nellore steers were used, and randomly assigned 30 minutes post-exsanguination to the following treatments: Achilles tendon suspension without ES (AT_NE; n=4); Achilles tendon suspension with ES (AT_ES; n=4); Pelvic suspension without ES (PS_NE; n=4); and Pelvic suspension with ES (PS_ES; n=4). ES was performed using an electro stimulator (model UFX7 NR-12; Fluxo Equipamentos Eletrônico Ltda; Chapecó, Santa Catarina, Brazil), set to apply an alternating current square wave (AC) at 15 Hz with a voltage of 300 V. The total application period was 78 seconds, consisting of 10 seconds of stimulation (pulse) followed by 3 seconds of rest, with 6 cycles total. Two electrodes were positioned, one at the Achilles tendon region and the other at the *Trapezius* muscle, ensuring the electrical current passes through the entire carcass. The pelvic suspension treatment consisted of hanging by the obturator foramen of the pelvic bone. At the time of deboning (24 h post-slaughter), a section of the *Longissimus* muscle between the 9th and 13th ribs and the *Biceps femoris* were removed; steaks were fabricated, vacuum packaged and aged for 0 and 14 days. After each period of aging the SF was analyzed according to the methodology proposed by [2]. The experimental design was a completely randomized 2 × 2 × 2 factorial arrangement (stimulated vs. non-stimulated; pelvic suspension vs. Achilles tendon suspension; 0 vs. 14 days of aging) with 4 replicates per treatment. The effect of the main factors on the SF was evaluated by analysis of variance using the MIXED procedure of SAS 9.3 software.

III. RESULTS AND DISCUSSION

For the *Longissimus*, an interaction was observed between the ES and the different suspension methods (p<0.001). Specifically, carcasses subjected to ES and suspended by the pelvic bone exhibited lower SF, resulting in a more tender cut (Figure 1). There was no statistical significance observed for the suspension method alone, nor was there an interaction between the treatment (ES or CON) and the aging time (0 or 14 days). Additionally, no interaction was found between the treatment, suspension, and aging time for the *Longissimus*.

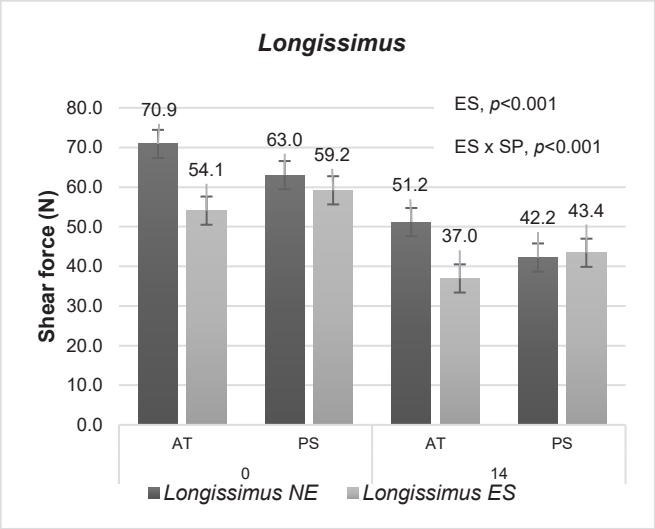

Figure 1. Shear force in the *Longissimus* of electrical stimulated (ES) and non-stimulated carcasses at 0 and 14 days of aging.

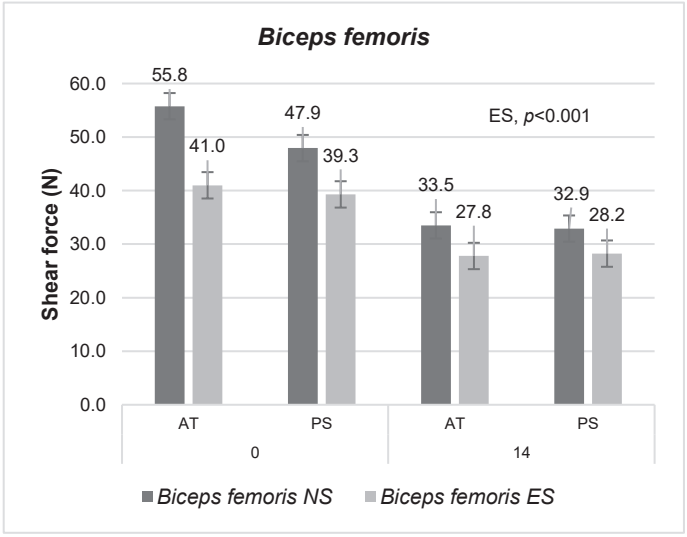

Figure 2. Shear force in the *Biceps femoris* of electrical stimulated (ES) and non-stimulated carcasses at 0 and 14 days of aging.

For the *Biceps femoris*, carcasses that underwent ES also effect (p<0.001) as measured by SF (Figure 2). There was no statistical significance for SF based solely on the suspension method. In both cuts, a significant effect of aging time was observed.

IV. CONCLUSION

For the *Longissimus* muscle, applying electrical stimulation and suspension by the pelvic bone were efficient to improve tenderness. In this same cut, it was observed that electrical stimulation alone also resulted in tender beef. The pelvic bone suspension method also resulted in greater tenderness in the *Biceps femoris*. Pelvic bone suspension may be more efficient when combined with carcass electrical stimulation, and beef tenderness is enhanced after a 14-day aging period.

ACKNOWLEDGEMENTS

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Feasibility of Hyperspectral Imaging as a tool for Quality Evaluation of Beef Burger Patties

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I. INTRODUCTION

Quality monitoring in added-value products, is a critical factor to achieve consistent production and transparency in the meat supply chain from farm to fork and ensure products are healthy and desired by consumers. Beef burgers are a major value added product typology in the beef sector with a globally high consumption pattern. Offline quality measurement of processed meat product quality attributes can be carried out using wet chemistry, sensory analysis etc. However, these methods are time consuming, destructive, and not adaptable to rapid or inline evaluation in an industry that is significantly sensitive to time. Technologies available for inline application inform on crude composition and contaminants but do not currently predict attributes reflective of process, technological, or sensory quality. The application of Industry 4.0 in the food sector would permit data-driven decisions that can help reduce batch rejection, and consequently food losses and waste, and increase consistency in quality and safety traceability [2]. The main objective of this study, therefore, is to investigate the feasibility of developing predictive models for quality attributes of raw and grilled beef burger patties, as a model system, using hyperspectral imaging and machine learning algorithms.

II. MATERIALS AND METHODS

An experimental design established variable burger patty formulations for quality prediction including fat content (5, 10, 15, 20, 25, and 30%), mincing levels (coarse and fine), and muscle types (round, brisket, and chuck steak). Figure 1 shows the workflow. Each batch of burgers (1 Kg) was prepared by chopping lean beef (95% VL) into approximately 20x20x20 mm pieces, then adding designated quantity of subcutaneous fat cubes along with 1% salt (1 g). The mixture was minced using a food mincer for 60 seconds. Coarse minced batches were prepared with 8 mm plate; whereas fine minced batches were made by passing the mixture first through the coarse plate(8 mm) and then a finer 3 mm plate. A hand burger former was used to form burgers of 90 mm diameter and 14 mm thickness. The total number of batches was 36 comprising 10 burgers for each batch. Quality attributes measured for raw burgers included water activity (a_w), Water Holding capacity (WHC). Samples were scanned, on both sides, using a NIR hyperspectral imaging (900-1700 nm), then stored at -18 °C until cooking. Each batch of burgers was grilled using an electric table grill for 15 minutes until the core temperature reached 75 °C, then scanned, and the cooking loss was calculated, followed by Texture Profile Analysis (TPA) using a texture analyzer. Compositional analysis (moisture content, fat, protein, and ash) was conducted following frozen storage. The Region of Interest (ROI) (i.e., the burger) was segmented, for each image, at each wavelength and the Mean Reflectance Spectra (MRS) as shown in Figure 2. MRS was calibrated using a standard white reference plate and the background (i.e., dark) images. Preprocessing techniques were applied to eliminate the effect of noise in the spectra. Prediction models were developed using Partial Least Squares Regression (PLSR), where data was divided into training (80%) and testing (20%) sets and 4-fold cross validation was applied on the training set, and the

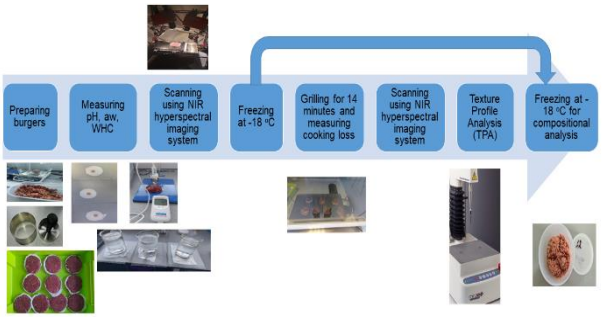

Figure 1. A schematic diagram of the workflow.

optimal training model was chosen based on the Root Mean Square Error of Cross Validation (RMSECV).

III. RESULTS AND DISCUSSION

Results of mean reflectance spectra for raw and grilled burgers, shown at Figure 3, revealed several absorption peaks around 960 nm and 1460 nm due to moisture content, and around 1200 nm due to the C- H stretching second overtone related to lipids. In general, high fat batches (i.e., lighter in color) showed lower absorbance than low fat batches (i.e., darker in color) which is comparable to results in [4]. Coarse patties showed more disperse spectra than fine patties due to the rougher surfaces of the former. Similarly, grilled patties showed more disperse spectra than raw ones. Predictive modeling of different quality attributes for the test set are shown in Table 1. The best PLSR models for raw burgers yielded r(RPD) values of 97.34%(3.34), 96.18%(3.62), and 81.55%(1.72) for moisture, fat, and protein, respectively. Whereas, grilled burgers showed less performance with r(RPD) values of 83.47%(1.83), 81.96%(1.75), and 80.62%(1.68) for moisture, fat, and cooking loss, respectively. These values are comparable to previous studies, which resulted r values of 97% for moisture and fat contents [5].

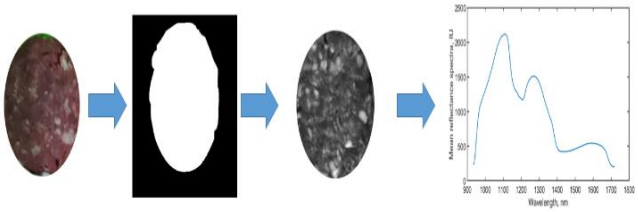

Figure 2. Procesisng of the hyperspectral images.

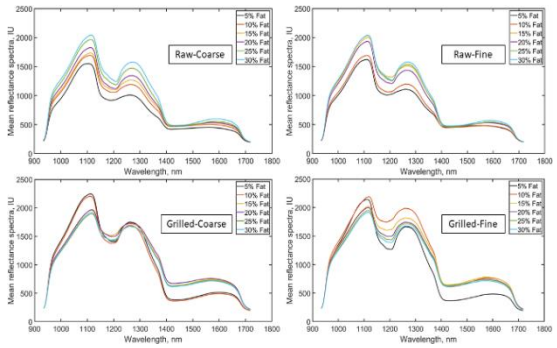

Figure 3. Results of MRS for raw and grilled burgers.

Table 1. PLSR results of different quality traits for raw and grilled burgers.

Burger status	Quality traits	Test set metrics*		
		r (%)	RMSEP	RPD
Raw	Moisture (%wb)	97.34	1.34	3.34
	Protein (%wb)	81.55	1.48	1.72
	Fat (%wb)	96.18	2.13	3.62
	Water holding capacity	64.40	10.60	1.30
Grilled	Moisture (%wb)	83.47	3.36	1.83
	Protein (%wb)	58.87	1.46	1.25
	Fat (%wb)	81.96	4.08	1.75
	Cooking loss (%)	80.62	3.75	1.68
	Gumminess, g	66.10	923.84	1.34
	Chewiness, g	69.69	1311.30	0.84
	Hardness, g	64.41	1355.30	1.19

IV. CONCLUSION

This study presented a feasible methodology for rapid and non-invasive quality assurance of nutrients and technological attributes of raw and cooked beef burgers using optical technology, offering a unit-based quality tracking. Results obtained in this study can be enhanced with feature selection as well as deep learning algorithms.

ACKNOWLEDGEMENTS

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SESSION 9

Meat Products Development

Thursday 22 August 2024

REFORMULATION OF GOAT BURGER USING AÇAÍ OIL HYDROGEL

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I. INTRODUCTION

Most meat products contain a high amount of saturated fat which can lead to cardiovascular and coronary diseases, atherosclerosis, obesity, and type II diabetes [1]. An alternative to reformulating these products and improving the fatty acid profile is to use healthier fats such as açaí oil which has good quality fatty acids, containing 70% oleic acid [2]. Hydrogel is a viable way of applying vegetable oils to meat products [3].

II. MATERIALS AND METHODS

The hydrogel was formed from a mixture of water, açaí oil, polysorbate 80 and guar gum. Five goat meat burguer treatments were developed with 0, 25, 50, 75 and 100% replacement of pork backfat with açaí oil hydrogel and frozen for 120 days. Moisture, protein, and ash determinations were carried out in accordance with AOAC [4]. Determination of lipid content followed the Bligh Dyer method [5] and lipid oxidation (TBARS) according to the method of Raharjo et al. [6]. For the analysis of water activity (a_w) the equipment was used Aqualab 4TE (METER Group Inc., Pullman, USA) and the pH was determined by the Hanna pH meter (Hanna Instruments, Woonsocket, USA). The color of the burgers was determined using Konica Minolta CR-400 colorimeter (Minolta Optics Inc. Konica, Japan). All analyses were carried out in triplicate and analyzed using ANOVA and the Tukey test through Statistica® 7.0 software with a 5% significance level.

III. RESULTS AND DISCUSSION

Table 1 shows the proximate analyzes of the burgers.

Table 1 – Proximate composition of burgers with the addition of açaí oil hydrogel.

(%)	Treatments				
	H0	H25	H50	H75	H100
Moisture	62.65±0.18c	65.06±0.06c	65.95±0.14c	69.24±0.15b	72.26±0.01a
Proteins	19.89±0.77	18.71±0.88	18.32±1.70	18.78±1.21	20.06±0.52
Lipids	12.12±0.30a	7.70±1.97b	8.22±1.14b	5.24±0.46b	1.85±0.85c
Ash	2.05±0.18	2.37±0.06	2.20±0.06	2.33±0.99	2.00±0.06

Means ± standard error in the same line followed by different lowercase letters indicate significant difference ($p<0.05$), according to the Tukey's test.

In the present study moisture increased as the percentage of hydrogel in the burgers increased, this may be since the guar gum, present in hydrogel formulation it is one hydrocolloid polysaccharide with ability to form solutions viscous in the presence of water, as it has nature hydrophilic. Protein, ash, and lipid values presented no significant differences ($p>0.05$).

The pH values were not affected by the reformulation ($p>0.05$), however there was a decrease during the 120-day storage period except for H0. a_w was not affected ($p>0.05$) by lipid replacement and was within the values established for this parameter.

Table 2 –Lipid oxidation (mg malonaldehyde /kg of meat) from burgers with fat replacement stored for 120 days.

	Days				
Treatment	0	30	60	90	120
H0	0.13 ± 0.01Bb	0.21 ± 0.05ABab	0.48 ± 0.21Aa	0.45 ± 0.02Aa	0.53 ± 0.13Aa
H25	0.17 ± 0.03ABb	0.26 ± 0.04Ab	0.44 ± 0.02Aa	0.34 ± 0.05ABa	0.42 ± 0.09Aa
H50	0.10 ± 0.02Bb	0.25 ± 0.03Aab	0.34 ± 0.03ABa	0.28 ± 0.03 Bab	0.38 ± 0.08ABa
H75	0.11 ± 0.07Bb	0.14 ± 0.02Bb	0.35 ± 0.01ABa	0.25 ± 0.08 Bab	0.30 ± 0.07Ba
H100	0.21 ± 0.03Ab	0.16 ± 0.03Bb	0.30 ± 0.04ABa	0.24 ± 0.04 Bab	0.34 ± 0.06Ba

Means ± standard error in the same line followed by different lowercase letters indicate significant difference ($p<0.05$), according to the Tukey's test. Means ± standard error in the same column followed by different uppercase letters indicate significant difference ($p<0.05$), according to the Tukey's test.

The açaí oil hydrogel reduced lipid oxidation of the burgers (Table 2). The H0 sample without hydrogel showed an increase of 307.70% in the TBARS content between day 0 and day 120, while the H100 sample showed an increase of 61.90%. One possible explanation for this is the presence of antioxidants in açaí oil, which contributed to lower fat oxidation.

Regarding the color of the burgers, on day 120 of storage, sample H100 was different from the control and showed one decrease in brightness which can be explained by the greater amount of replacement with açaí oil hydrogel. The attribute a* decreased and b* increased during the storage period.

IV. CONCLUSION

Through the analyzes it was possible to conclude that the replacement with açaí oil hydrogel was viable and reduced lipid oxidation.

ACKNOWLEDGEMENTS

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INSECTS (*Acheta domesticus and Tenebrio molitor*) POWDER AS PARTIAL MEAT REPLACER IN FRANKFURT-TYPE SAUSAGES

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I. INTRODUCTION

Edible insects have gained relevance in last years because of their high nutritional value (source of proteins and unsaturated fatty acids) and sustainable production (low emissions of greenhouse gases and reduced use of natural resources) [1], in line with the goals required by the agrifood chain for a more efficient and sustainable food production. It is worth noting that, although international organizations defend the benefits of entomophagy, attitudinal barriers still persist in Western societies [2]. It is thought that this rejection of insect consumption will be lessened if they are incorporated into the diet not as such, but in the form of powders for use as food ingredients. In this way, the aim of this work was to evaluate the feasibility of including powders from edible insects (*Acheta domesticus* and *Tenebrio molitor*) as partial beef meat replacers in cooked sausages (Frankfurt-type) and their effect on their nutritional, technological, and sensory properties.

II. MATERIALS AND METHODS

Frankfurt-type sausages with 65% beef meat and 35% pork backfat (100% meat); and 20% water, 2.5% sodium chloride, 300 mg/kg sodium tripolyphosphate, 150 mg/kg sodium nitrite, 2 g/kg power smoke and 0.3% spices, were elaborated following a traditional procedure. Insect powders (TM: *T. molitor* and AD: *A. domesticus*) were used for the 7.5% (7.5%TM and 7.5% AD) and 15% (15%TM and 15% AD) beef meat replacement. Proximate composition, emulsion stability (total expressible fluid; TEF (%)), pH, and CIELAB color properties were assessed. Consumer panelist scored the samples for flavor, taste, hardness, juiciness, color and overall appearance. Significant differences were determined by means of ANOVA and Tukey test ($p<0.05$).

III. RESULTS AND DISCUSSION

Table 1 shows the proximate composition and technological properties of sausages with insect powders. The partial replacement of beef meat by insect powders resulted in sausages with lower moisture and higher protein than control ($p<0.05$). The protein content was higher when AD powder was added than in the case of TM, what is related to the protein content in both powders [3]. Sausages added with TM powder showed higher fat content than control but this content was lower when AD powder was used ($p<0.05$) which can be attributed to the fat content of both powders [3]. Not only is important the fat content but the fatty acid profile, and it has been reported that insect powders have higher polyunsaturated fatty acids content than beef meat. The use of insect powders increased the emulsion stability, without differences between the type of powder used but dependent on the concentration; the higher the replacement percentage, the higher the emulsion stability ($p<0.05$). The addition of insect powders resulted in sausages with less lightness (dependent on powder concentration but not on powder type) and redness (lower redness when AD powder was used) than control.

Table 1 – Proximate composition and physicochemical properties of sausages with insect powders.

Sample	Control	7.5%TM	15%TM	7.5%AD	15%AD
Moisture (%)	70.93±0.29^{a}	66.76±0.02^{b}	64.93±0.55^{c}	67.63±0.47^{b}	65.57±0.31^{c}
Ash (%)	1.82±0.07^{a}	1.91±0.15^{a}	1.86±0.14^{a}	1.95±0.23^{a}	1.71±0.65^{a}
Fat (%)	9.24±0.43^{b}	10.23±0.44^{a}	10.10±0.39^{a}	8.41±0.52^{c}	8.13±1.09^{c}
Protein (%)	16.15±0.10^{c}	16.38±0.09^{c}	17.31±0.23^{b}	17.79±0.25^{b}	19.19±0.37^{a}
TEF(%)	14.76±2.45^{a}	9.57±1.01^{b}	2.98±0.58^{c}	11.90±2.14^{ab}	3.77±1.71^{c}
pH	6.02±0.03^{d}	6.10±0.02^{c}	6.28±0.02^{a}	6.15±0.02^{b}	6.16±0.01^{b}
L*	58.45±2.16^{a}	55.27±3.09^{b}	54.37±1.71^{c}	55.69±1.39^{b}	51.70±1.13^{c}
a*	7.34±0.56^{a}	6.32±0.49^{b}	5.82±0.83^{c}	5.40±0.40^{c}	5.60±0.24^{c}
b*	8.38±0.23^{c}	10.21±0.75^{a}	10.17±1.28^{b}	8.56±0.56^{ac}	8.38±0.38^{c}

$^{a-c}$: different letters in the same row indicate significant differences among formula.

The used of insect powders (TM and AD) for the beef meat replacement at 7.5% resulted in sausages with good sensorial acceptance (all the attributes evaluated were scored higher than 5) (Figure 1). Higher substitution levels (15%) resulted in sausages with low scores, mainly in overall taste and flavor when TM powder was used, and even in appearance, juiciness and color for AD powder.

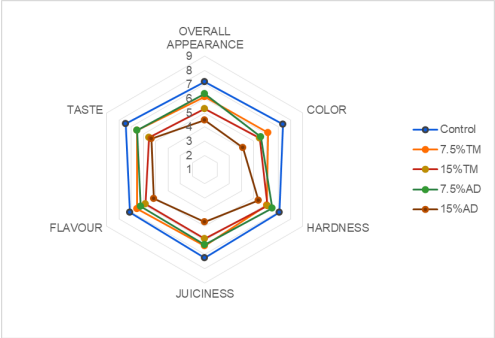

Figure 1. Sensory properties of Frankfurt-type sausages with insects powder as partial meat replacer

IV. CONCLUSION

The use of insect powders for the partial replacement of beef meat in Frankfurter-type sausages is a technologically viable option that opens the door to their use as a protein source in the development of more sustainable meat products, although its negative effect on some sensory characteristics still needs to be improved.

ACKNOWLEDGEMENTS

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Effects of whey protein and its hydrolysate on the qualities of cured sausage

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I. INTRODUCTION

Population growth and fluctuating crop yields due to climate change have worsened food problems such as poor-quality food and food shortages, and malnutrition and hunger become a global problem. In addition, the growing health consciousness has led to an increased demand for functional foods. Therefore, it is essential to develop meat products with added health and nutritional value. Whey is a byproduct of cheese production and is generated after casein is coagulated in the cheese-making process, which is rich in protein and has a high nutritional value. Whey protein hydrolysates (WPH), produced by enzymatic degradation of whey proteins (WP), have shown utility in food processing. It has also been reported that WPH contains bioactive peptides [1]. This study focused on WP and WPH, and aimed to assess their impact on sausage quality.

II. MATERIALS AND METHODS

Commercially obtained WP concentrate (Daiichirakuto EM-20; Daiichi Kasei Co., Ltd., Kyoto, Japan) and WPH (WPH; Daiichi Kasei Co., Ltd.) were used in all the performed tests. To prepare the WP and WPH ethanol (EtOH) extracts, the WP and WPH were suspended in 70% EtOH overnight at 4 °C. The suspension was centrifuged, filtered, and dried using a rotary evaporator. The dried samples were resuspended in distilled water and lyophilized to prepare the extracts. Fe^{3+} reduction was measured using a previously described method [2]. The oxidation-reduction potential (ORP) values were measured using an ORP meter (Kasahara Chemical Instruments Corp.). For the NO_2^- reducing activity, the residual NO_2^- level was determined according to a previously reported method [3], and the decrease ratio from the initial sodium nitrite concentration was determined as follows: decreasing ratio (%) = ((Ac – As) / Ac) × 100. Ac and As are the absorbance of the control and sample, respectively.

To prepare the tested sausage model, 2% NaCl, 30 ppm $NaNO_2$, and WP or WPH were added to ground pork and mixed thoroughly on ice using a mortar. After mixing, the samples were packed in sanitary plastic bags and heated at 75 °C for 20 min in a water bath (TAITEC, Koshigaya, Japan). The color of the sausage model was evaluated using a spectrophotometric colorimeter (Konica Minolta Sensing, Inc., Tokyo, Japan). The measurement was carried out in reflectance. Also, the observer angle and the light source were used 10° and D65 light, respectively. The sausage model was heated to 78°C in a water bath, and the decanted liquid and cooking loss were calculated as the weight lost during heating as a percentage of the initial weight: [100 × (initial weight − final weight) / initial weight]. A blind sensory test was also conducted to evaluate the palatability of the tested sausage model among the participants.

III. RESULTS AND DISCUSSION

As shown in Table 1, the Fe^{3+} reducing activity of the WPH EtOH extract was significantly higher than that of the WP EtOH extract at 25 and 50 mg/mL ($p < 0.05$). In addition, all ORP values of the WPH EtOH extract were significantly lower than those of the WP EtOH extract at the same contents ($p < 0.05$), and the NO_2^- decreasing ratio of the WPH EtOH extract at all levels was significantly higher than that of the WP EtOH extract ($p < 0.05$). Thus, the WP and WPH EtOH extracts exhibited

antioxidant and reducing activities [4]. As shown in Figure 1, the *a** value of 5.0% WPH EtOH extract supplemented sausage was significantly higher than that of 5.0% WP EtOH extract supplemented and control sausage ($p < 0.05$). The *L** and *b** values of 5.0% WPH EtOH extract supplemented sausage tended to be lower than those of 5.0% WP EtOH extract supplemented sausage. Additionally, the water cooking loss of 5.0 % WPH EtOH supplemented sausage was significantly suppressed compared to that of the 5.0% WP EtOH supplemented and control sausages ($p < 0.05$). Moreover, the WPH supplemented sausages demonstrated a high score in the flavor category of the palatability test. Therefore, WPH is expected to have improved the quality of cooked sausages.

Table 1 – Antioxidative and reducing activities of WP and WPH EtOH extracts.

Concentration (mg/mL)		Fe^{3+} reduction (umol/L: Trolox equivalent value)	ORP value (mV)	NO_2^- decreasing ratio (%)
WP EtOH extract	10	11.24 ± 8.74	228.0 ± 15.7	29.86 ± 5.80
	25	17.56 ± 12.32	210.0 ± 5.6	27.77 ± 7.08
	50	31.25 ± 25.35	206.5 ± 13.9	22.62 ± 6.29
WPH EtOH extract	10	27.79 ± 11.98	181.3 ± 5.7 *	42.08 ± 6.89 *
	25	63.05 ± 11.64 *	168.5 ± 9.3 *	40.82 ± 2.03 *
	50	104.87 ± 12.32 *	160.0 ± 15.9 *	33.95 ± 5.00 *

*: p<0.05 vs the value of WP-EtOH at the same concentration

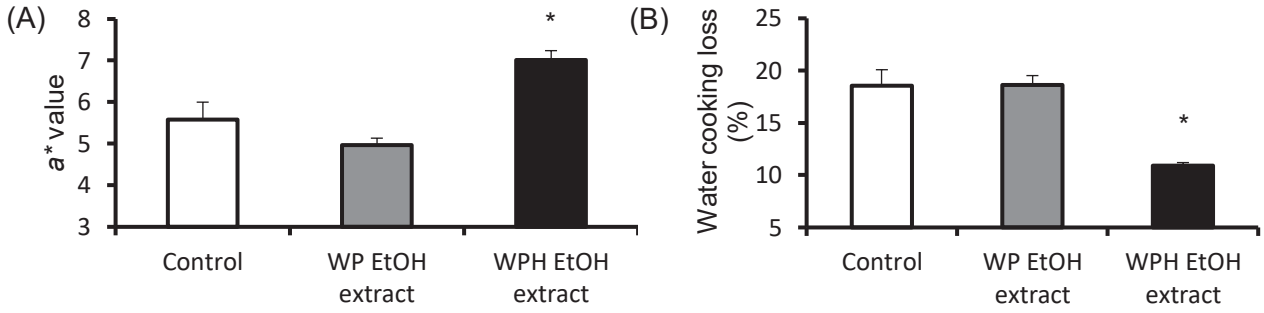

Figure 1. Redness and water holding capacity of sausages added WPH EtOH extracts.

(A) and (B) show *a** value and water cooking loss of sausage models, respectively. *: p<0.05 vs the control and WP

IV. CONCLUSION

In conclusion, this study demonstrated that WPH extracts not only showed antioxidative and/or reducing activities but also improved the quality of cooked sausages. WPH is known to have high potential for nutritional supplementation. In meat processing, WPH contributes not only to nutritional supplementation but also to the addition of bioactivities (such as antioxidant and reducing actions) and improves the quality of meat products.

ACKNOWLEDGEMENTS

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COOKING SHRINKAGE IN PLANT-BASED AND MEAT PATTIES

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I. INTRODUCTION

When assessing meat, consumers typically consider three pivotal moments: the initial purchase, where factors like appearance and color hold significant sway; the cooking process, during which shrinkage and loss of juices are scrutinized; and finally, consumption, where tenderness, juiciness, and aroma reign supreme. These parameters are intricately linked, with a discernible correlation between shrinkage and juiciness [1]. The perception of meat shrinkage during cooking is often equated with inferior quality [2], however, when examining plant-based products, such as patties, shrinkage is less conspicuous [3]. Plant-based meat analogue foods are meticulously crafted to replicate the characteristics of their animal-based counterparts, including patties, sausages, and nuggets but in plant-based products. This study endeavors to compare the shrinkage observed in cooked meat patties with plant-based meat analogue patties.

II. MATERIALS AND METHODS

A total of 146 patties were utilized, divided into eight distinct types. These consisted of six homemade types (HB, 64 patties divided between the 6 groups), one commercial type (CB, 30), one commercial precooked (PB, 22) pea protein-based type of patty, and one homemade meat patty (MB, 30) blending 60% beef and 40% pork. The 6 HB types were crafted by combining various proportions of commercial pea protein products, and diverse preparation methods and recipes. Evaluation of the patties took place post-cooking. Cooking shrinkage was gauged using a 1 cm thick disc, employing the methodology outlined by Barbera and Tassone [4], expressed as a percentage of the raw area. Other parameters were measured following the protocol outlined by Mabrouki et al. [5] such as: total moisture content measured on frozen sample as percentage of the raw weight (RW); fluid and protein content in the cooked sample, expressed as a percentage RW. Additionally, four Texture Profile Analysis parameters - hardness, gumminess, chewiness, and adhesiveness - were assessed on each homogenized cooked sample [5]. The specific density of the homogenized cooked sample was also determined. Statistical analyses, including Variance and Canonical Discriminant Analysis (CDA), were conducted using SAS 9.4 and Rstudio to compare the MB, CB, PB types and HB group. To facilitate comparisons, the 6 HB types have been consolidated into a single group.

RESULTS AND DISCUSSION

Table 1 presents the average values of the parameters examined across different patty types. It was observed that the MB exhibited the most substantial shrinkage, while both the CB and HB demonstrated comparable rates of shrinkage, and the PB exhibited the least. Remarkably, although the MB and PB boasted the highest protein content, and the HB had the lowest, the latter paradoxically displayed a higher fluid-to-mouth value. This observation suggests that veggie patties may indeed be juicier than their meat counterparts. However, an analysis of density reveals that the MP has a significantly higher value compared to the plant-based patties, indicating a higher liquid content. This disparity stems from the distinct protein sources - animal *versus* vegetable. Animal proteins tend to form a more robust lattice structure that effectively retains liquids, a characteristic not typically seen with plant proteins. Further measurements using Texture Profile Analysis (TPA) confirm these differing behaviors, with the meat patty exhibiting greater hardness even surpassing that of the pre-cooked PB patty. This distinct response of the meat patty compared to plant-based alternatives aligns with findings from other studies [6,7], albeit with varying numerical values due to differences in methodology. The meat patty experiences more significant shrinkage and presents as harder and chewier compared to plant-based counterparts. Moreover, when considering MB fluid to mouth value on a raw weight, there

Table 1 – Average values measured on cooked samples, except total moisture on frozen one (n = 146).

Traits		Type of patty				MSE
		MB	CB	HB	PB	
Cooking shrinkage	%	24.2^{A}	11.7^{B}	11.0^{B}	$7,1^{C}$	4.433
Total moisture	% WB	63.7^{A}	60.3^{B}	59.9^{B}	54.4^{C}	6.592
Fluid to the mouth	% WB	43.7^{B}	46.3^{A}	46.7^{A}	46.0^{A}	5.241
Density	mg/mm^3	1.36^{A}	1.00^{C}	1.03^{C}	1.10^{B}	0.0051
Crude Protein	% WB	20.8^{B}	18.6^{C}	15.9^{D}	23.1^{A}	3.223
Hardness	N	28.6^{aA}	5.6^{C}	10.1^{B}	25.1^{bA}	9.975
Gumminess	N	13.9^{A}	2.3^{D}	5.7^{C}	10.2^{B}	4.265
Chewiness	N	10.9^{A}	2.1^{D}	5.1^{C}	8.3^{B}	2.298
Adhesiveness	10^{-3} J	-8.2^{C}	3.0^{B}	7.7^{A}	-9.0^{C}	0.023

MB = meat patty; CB = commercial plant-based patty; HB = homemade plant-based patties; PB = precooked commercial plant-based patty, RW = raw weight. $^{a, b}$ P = 0.05, $^{A, B, C, D}$ P =< 0.01: based on Tukey's test on the same row.

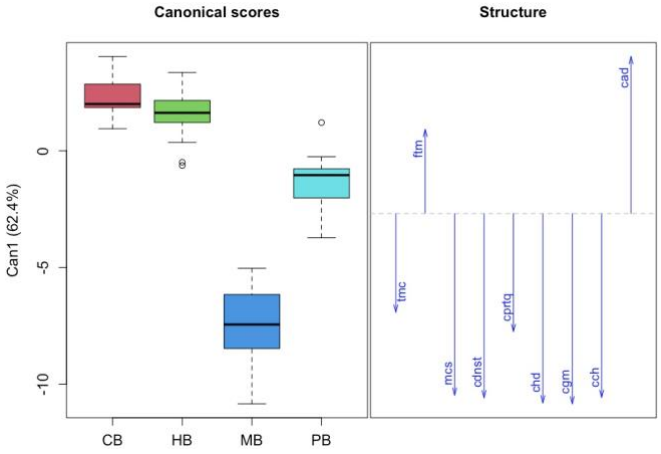

Figure 1. Canonical Discriminant Analysis and contribution of parameters, according to the Total Canonical Structure, to separate the different types of patties. CB commercial, HB homemade, MB meat and PB pre-cooked patties. Tmc - total moisture content; ftm - fluid to the mouth; mcs – cooking shrinkage; cprtq - raw protein; chd – hardness; cgm – gumminess; cch - chewiness; cad – adhesiveness; cdns – density.

appears to be a higher quantity of water within a smaller volume, as indicated by the density measurements. This aspect has been overlooked in prior literature.

Figure 1 provides a visual summary of a CDA, highlighting the contribution of various parameters in identifying distinct patty groups, notably showcasing the meat patty's divergence from the others.

CONCLUSION

Our study highlights the crucial role of cooking shrinkage in shaping vegetable patties to closely mimic the texture and protein attitude of their meat counterparts.

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BOUGAINVILLEA SPECTABILIS AS A POTENTIAL ALTERNATIVE TO NITRITES IN COOKED HAM ELABORATION

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I. INTRODUCTION

Nitrites (NO_2^-) and nitrates (NO_3^-) are commonly used additives aimed at enhancing meat quality. They play an important role by providing an attractive color to meat, enhancing flavor, contributing to the antioxidant properties, and improving antimicrobial characteristics. However, the International Agency for Research on Cancer (IARC) has stated that red and processed meat consumption is associated with cancer risk [1]. Thus, in the last decades, an effort has been made to explore additive alternatives to reduce the use of nitrites in processed meat. In this work the use of *Bougainvillea spectabilis* as an alternative to nitrites in ham is proposed. This flower, commonly used in Mexico as a natural remedy to address respiratory diseases [2], contains compounds such as flavonoids, alkaloids, phenols, and tannins, which contribute to its potential as a natural additive in meat products [2-5].

II. MATERIALS AND METHODS

Three different drying methods were evaluated to obtain the additives from *Bougainvillea spectabilis* bracts and flowers: air drying (BA), foam-mat drying (BF), and oven drying (BO). In foam-mat drying, the flowers were mixed with albumin, maltodextrin, hydroxyethyl cellulose, and Tween-80 as foaming agents and stabilizers, and then dried by application of hot air. Five cooked ham formulations composed by pork (71%), polyphosphates (0.5%), dextrose (0.7%), carrageenan (0.75%), sodium erythorbate (0.05%), and bougainvillea were designed. An injection of 40 % was applied and formulations were cooked to reach 69 °C core temperature. F1 batch contained 0.01% of BF, F2 batch 0.05% of BF, F3 0.25% of BF, F4 0.1% of BA, and F5 0.1% of BO. Two control batches were considered, one containing nitrites (Control NO) and the other without (Control). Each batch was divided into three equal portions, which were vacuum packed and stored at 4°C. Evaluation of meat antioxidant properties, color and organoleptic characteristics were conducted at weeks 0, 4 and 8. The evaluation of the antioxidant profile of the ham was carried out by DPPH, ABTS, and FRAP methodologies. Lipid oxidation was evaluated following the development of thiobarbituric acid reactive substances (TBARS). Nitrite content was determined by the Griess test with slight modifications [7]. Moisture content in ham was measured and CIEL*a*b* parameters were determined. Sensory evaluation of the samples was performed using a 5-point hedonic test conducted by twenty-one trained panelists. Hedonic scores ranged from 1 to 5 from very unpleasant (1) to excellent (5). The test included the evaluation of color, odor, texture, taste, and overall acceptability. Statistical analysis of the data obtained was performed using Minitab 17 software.

III. RESULTS AND DISCUSSION

Three bougainvillea powders were elaborated to provide the reddish color while offering antioxidant and antimicrobial properties to cooked ham as an alternative to nitrite salts (Figure 1). The addition of bougainvillea powders equaled or improved the antioxidant capacity in ham formulations without the presence of nitrite salts in comparison with the nitrite control. This antioxidant effect was affected by storage.

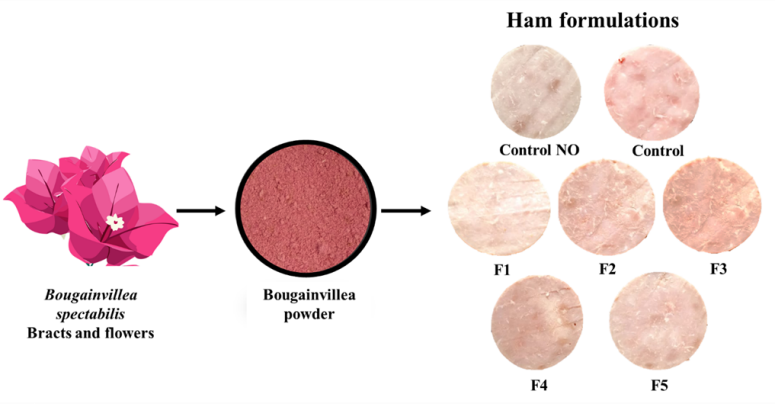

Figure1. *Bougainvillea spectabilis* powder and ham formulations

Nevertheless, the formulations containing the additives continued presenting higher scores of antioxidant activity compared to the control samples. After cold storage, this trend remained. The addition of bougainvillea additives in the formulation prevented the oxidation process of lipids determined by TBARs during storage compared with the control samples. The incorporation of the bougainvillea in ham did not significantly change the moisture content or pH ($p > 0.05$). Sensory evaluation of the ham was carried out on week 0, 4 and 8 of storage. The panelists gave the highest scores to the F4 formulation. After 8 weeks of storage (4°C) no changes in color were appreciated. Also, no unpleasant odors or flavors were perceived.

IV. CONCLUSION

To the best of our knowledge, this is the first work to propose the use of *Bougainvillea spectabilis* as an antioxidant additive in ham. Evaluation through DPPH, ABTS, and FRAP assays demonstrated the efficacy of bougainvillea in mitigating oxidation during storage, enhancing sensory qualities. Among the drying methods investigated, air drying exhibited superior antioxidant and sensory effects. Ham formulations presented a high score in overall acceptability during the sensory evaluation. *Bougainvillea* presents potential to replace the use of nitrite salts in ham elaboration.

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PHYSICAL-CHEMICAL INFLUENCE OF *ACHETA DOMESTICUS* FLOUR AS A PARTIAL REPLACEMENT FOR BEEF IN BURGERS

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I. INTRODUCTION

Insect farming demonstrates good prospects for food and animal feed due to high fertility and growth rates, efficient feed conversion, ability to thrive in reduced spaces, resistance to drought, lower risk of transmitting zoonotic diseases, and more eco-friendly footprints compared to conventional livestock [1]. Nutritionally, insects are sources of high-quality proteins, unsaturated and essential fatty acids, fiber, vitamins, and minerals [2]. In this sense, consuming edible insects (entomophagy) is pointed out as a contributor to addressing global food supply shortages, combating food insecurity, while also presenting beneficial environmental, nutritional, and subsistence appeals [3]. Consumers, especially in Western countries, are still hesitant to include insects in their diet mainly due to food neophobia, and spreading reliable information about the benefits of entomophagy can help overcome this challenge [4]. Therefore, this study aimed to develop burgers containing cricket (*Acheta domesticus*) flour (ADF) as a partial replacement for beef and evaluate the influence on some of the main limitations of their quality, lipid oxidation and color of the raw and cooked burgers (ready-to-eat).

II. MATERIALS AND METHODS

ADF was imported from a local market in Italy. Three different treatments were made to achieve isoprotein formulations considering that the meat forequarter contained 20.5% protein, while ADF presented 64.0% (as determined previously): control treatment with 75.0% beef (CON), treatment with a 10% reduction in the amount of beef (T10: 67.5% beef + 2.4% ADF), and treatment with a 20% beef reduction (T20: 60% beef + 4.8% ADF). All treatments had pork backfat (20.0 g/100.0 g) and 3% hamburger seasoning, and formulations were completed with ice-cold water. The meat and pork backfat were ground on a 4 mm disc (Picador 22, Beccaro). The ingredients were mixed until they formed a homogeneous mass. The burgers were shaped into approximately 100-gram units using a manual formatting machine (HP112, Picelli). The samples were stored frozen (-18ºC) and thawed at 4ºC for 24 hours before analysis. To assess the cooked burger samples, they were cooked at 180ºC on an electric heating plate until the geometric center reached 75ºC. Objective color (L: brightness; a*: green-red; b*: blue-yellow) was evaluated using a portable colorimeter (MiniScan XE Plus, HunterLab) with the D65 standard illuminant and observation angle of 10°. Cooked burgers (CB) were cut longitudinally to assess their internal color. Raw burgers (RB) and CB were evaluated for lipid oxidation using the thiobarbituric acid reactive substances (TBARS) method [5]. Statistical analysis was performed by Analysis of Variance (ANOVA) and Tukey's test within the significance level of 5%, using SAS© software version 9.4 (SAS Institute Inc., North Carolina, USA). The experiment followed a completely randomized design with three treatments and two replications at the processing level.

III. RESULTS AND DISCUSSION

Table 1 presents the results of lipid oxidation and color parameters. Adding a greater amount of cricket flour led to an increase in lipid oxidation in both RB and CB, while T10 did not differ from CON. Some authors establish 2.0 mg MDA/kg as the limit for the perception of rancidity by consumers, therefore, all ready-to-eat burgers are tolerable [6].

When ADF was added as a replacement for meat, it caused an increase in luminosity and a reduction in the red index in RB samples. This could be due to the color characteristics of ADF (L = 57.54±0.99;

a* = 5.46±0.14; b* = 18.06±0.67) compared to ground beef (L = 46.40±0.96; a* = 16.55±0.95; b* = 14.49±1.03). After cooking, the difference in both L and a* parameters decreased between the three treatments, and T10 showed similar luminosity to CON, but slightly lower a*, also noticeable concerning T20 ($P<0.05$). Regarding yellowness (b*), there was no difference between all treatments before or after cooking ($P>0.05$). The RB and CB samples are shown in Figure 1.

Table 1 – Parameters of objective color and lipid oxidation of burgers with and without the addition of FAD.

Parameters	Cooking	Treatment		
		CON	T10	T20
TBARs (mg MDA/kg sample)	Raw	0.62 ± 0.13^{b}	0.68 ± 0.07^{b}	0.95 ± 0.27^{a}
	Cooked	1.23 ± 0.07^{b}	1.32 ± 0.09^{b}	1.66 ± 0.21^{a}
L	Raw	52.13 ± 1.24^{c}	53.58 ± 0.36^{b}	54.94 ± 0.50^{a}
	Cooked	48.27 ± 1.26^{a}	48.77 ± 0.38^{ab}	46.94 ± 0.60^{b}
a*	Raw	15.82 ± 0.72^{a}	11.96 ± 0.61^{b}	8.01 ± 0.32^{c}
	Cooked	6.11 ± 0.40^{a}	5.48 ± 0.19^{b}	5.20 ± 0.27^{b}
b*	Raw	13.33 ± 0.80	13.93 ± 0.63	12.86 ± 0.40
	Cooked	12.13 ± 1.69	13.08 ± 0.68	12.47 ± 0.98

Means ± standard deviation. $^{a-c}$ different lowercase letters on the same line (different treatments): values differ statistically from each other ($P<0.05$); no superscript letters: no significant difference ($P>0.05$). Treatments: CON: without adding insect flour; T10: 10% beef reduction; T20: 20% beef reduction.

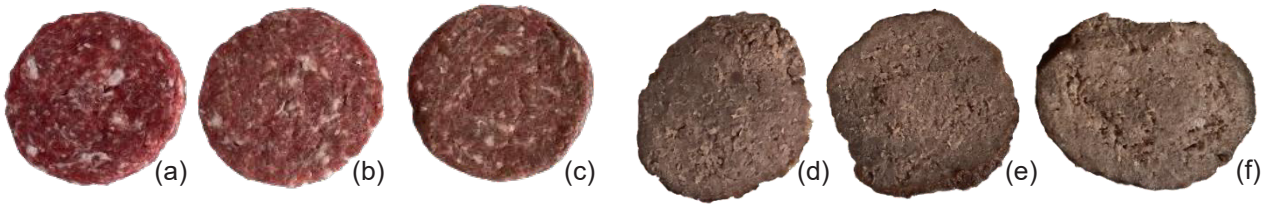

Figure 1. Typical appearance of samples. RB: (a) CON; (b) T10; (c) T20; Internal CB: (d) CON; (e) T10; (f) T20.

IV. CONCLUSION

The study showed that using *Acheta domesticus* as a substitute for beef in burgers can be a feasible option with minimal or no alteration in the investigated physical-chemical properties, particularly when 10% of the meat content was replaced (T10). This finding can be considered promising for offering more ecologically sustainable protein alternatives to address the combat of food insecurity.

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Revealing Nutrient Composition and Fatty Acid Diversity in Hybrid Products Combining Meat and Plant Protein

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I. INTRODUCTION

Consumers are increasingly interested in shifting towards diets with lower consumption of animal products (flexitarian) [1]. Reducing meat consumption is easier than completely excluding it from the diet by becoming vegetarian or vegan. Hence, hybrid meat products, in which a portion of the meat is replaced by sources of more sustainable protein, are highly attractive. This may bridge the gap between meat and meat-free products, allowing consumers to maintain their conventional use of food products [2]. The aim of the study was to investigate the effect of adding plant-based proteins on the fatty acid profile of hybrid meat products.

II. MATERIALS AND METHODS

Texturized pea protein (TPP 67C) was sourced from Agri Food Ingredients (N. G. Alexander & Co Pty Ltd, Melbourne, Australia). The TPP was rehydrated by mixing it with water at 90°C for 5 minutes at a 1:2 ratio (TPP: water, by weight), and subsequently cooled to room temperature (23°C ±1°C). The beef was coarsely ground using a mincer with a 4 mm diameter plate. The beef mixture was kneaded with salt, pork fat, and TPP for one minute each. After refrigeration, the meat patties were shaped into 150 g portions using a 10 cm patty maker. The composition of the hybrid formulas was as follows: Beef 61%; Hydrated texturized pea protein 20%; Pork fat 4%; Water 14.5%; Salt 0.5%. The composition of the control formulas was: Beef 81%; Hydrated texturized pea protein 0%; Pork fat 4%; Water 14.5%; Salt 0.5%. Three patties were selected randomly to test their chemical composition. Determination of crude protein followed the Leco Dumas method described in AOAC 992.15. A moisture, fat, and ash content were determined according to AOAC protocol (fat 920.39; moisture 925.09, ash AOAC 923.03). Determination of fatty acids composition of samples carried out according to the method described by Hastie et al. [3]. Statistical analyses were conducted using the statistical package PQStat version 1.8.4.152. The results in both groups were compared using the Student t-test for independent groups. A test probability of $p<0.05$ was considered significant, while a test probability of $p<0.01$ was considered highly significant.

III. RESULTS AND DISCUSSION

The proximate composition and fatty acid profile of the hybrid and control patties are shown in Table 1.

Table 1. The proximate composition and fatty acid profile of the hybrid and control patties

	Control product	**Hybrid product**
Water [%]	$74.52^{a}\pm0.24$	$73.18^{a}\pm0.18$
Protein [%]	$17.00^{b}\pm1.03$	$14.83^{a}\pm0.48$
Fat [%]	$7.38^{b}\pm0.47$	$5.51^{a}\pm1.18$
Ash [%]	$1.28^{a}\pm0.07$	$1.03^{a}\pm0.09$
Fatty acid profiles [%]		
Butanoic acid (C4:0)	$0.0091^{a}\pm0.001$	$0.0107^{b}\pm0.001$
Hexanoic acid (C6:0)	$0.0244^{a}\pm0.001$	$0.0528^{b}\pm0.001$
Heptanoic acid (C7:0)	$0.0702^{a}\pm0.003$	$0.1074^{b}\pm0.001$
Capric acid (C10:0)	$0.4818^{a}\pm0.034$	$0.4724^{a}\pm0.005$
Undecanoic acid (C11:0)	$0.0097^{a}\pm0.001$	$0.0093^{a}\pm0.001$
Lauric acid (C12:0)	$0.6233^{a}\pm0.029$	$0.6157^{a}\pm0.012$
Tridecanoic acid (C13:0)	$0.0398^{a}\pm0.001$	$0.0393^{a}\pm0.001$
Myristic acid (C14:0)	$7.4517^{b}\pm0.147$	$7.0235^{a}\pm0.096$
Myristoleic acid (C14:1)	$0.2529^{b}\pm0.002$	$0.2108^{a}\pm0.006$
Pentadecanoic acid (C15:0)	$1.1689^{b}\pm0.010$	$1.0927^{a}\pm0.018$
Palmitic acid (C16:0)	$40.6200^{b}\pm0.092$	$39.1601^{a}\pm0.183$
11-Hexadecanoic acid (C16:1)	$2.3144^{b}\pm0.026$	$2.1673^{a}\pm0.008$

Cis-10-heptanoic acid (C17:1)	0.8367^{b}±0.002	0.754^{a}±0.010
Stearic acid (C18:0)	27.1786^{b}±0.247	25.8146^{a}±0.193
Oleic acid (C18:1)	4.8115^{b}±0.014	4.6224^{a}±0.024
Linoleic acid (C18:2)	6.6336^{a}±0.048	8.6588^{b}±0.060
Linolenic acid (C18:3 n-6)	2.5997^{a}±0.022	3.4444^{b}±0.016
Linolenic acid (C18:3 n-3)	1.5855^{a}±0.008	2.4295^{b}±0.002
Arachidic acid (C20:0)	0.685^{a}±0.002	0.8037^{b}±0.015
11-Eicosanoic acid (C20:1 n-9)	0.6803^{b}±0.004	0.6640^{a}±0.001
cis-11,14-Eicosanoic acid (C20:2 n-6)	0.3364^{a}±0.001	0.3393^{a}±0.006
Heneicosanoic acid (C21:0)	0.0155^{a}±0.001	0.0239^{b}±0.001
Arachidonic acid (C20:4 n-6)	1.1438^{b}±0.008	0.9048^{a}±0.016
11,14,17-Eicosatrienoic acid (C20:3)	0.1470^{a}±0.005	0.1520^{a}±0.003
Decosanoic acid (C22:0)	0.0883^{a}±0.001	0.1203^{b}±0.002
13-Decosanoic acid (C22:1 n-9)	0.0227^{a}±0.001	0.0426^{b}±0.002
13,16-dcocasadienoic acid (C22:2)	0.0670^{a}±0.006	0.0637^{a}±0.008
Tricosanoic acid (C23:0)	0.0054^{a}±0.003	0.0037^{a}±0.002
Tetracosanoic acid (C24:0)	0.0162^{a}±0.008	0.1015^{b}±0.001
4,7,10,13,16,19-Docosahexaenoic acid (C22:6 n-3)	0.0955^{b}±0.001	0.0798^{a}±0.001
Sum of Saturated Fatty Acid (SFA)	**75.34^{b}±8.22**	**72.53^{a}±7.93**
Sum of Monounsaturated Fatty Acid (MUFA)	**8.92^{b}±0.80**	**8.46^{a}±0.78**
Sum of Polyunsaturated Fatty Acid (PUFA)	**21.53^{a}±1.12**	**24.53^{b}±1.78**

Results are expressed as mean ± standard deviation. Different lettering in the rows indicates significant differences $p<0.05$

The protein and fat content of the hybrid products are lower than that of the control samples. Furthermore, replacing meat proteins with pea protein isolate significantly modifies the fatty acid profile of the resulting products. The content of polyunsaturated fatty acids was higher in hybrid products compared to the control group (21.53% vs. 24.53%, respectively), while the content of saturated fatty acids was higher in the control group (75.34% for the control, 72.53% for the hybrid). Plant protein sources generally have a higher polyunsaturated fatty acid (PUFA) content compared to animal-derived products [4]. Consequently, the incorporation of pea protein led to a notable increase in PUFA content compared to the control samples. Completely different results were obtained by Flores et al. [5], who added coconut oil to hybrid meat patties as a fat substitute. They found an increased saturated fatty acid content while decreasing the content of mono- and polyunsaturated fatty acids compared to the meat product.

IV. CONCLUSION

In conclusion, hybrid products have a more favorable fatty acid profile than conventional meat products. The exception, however, could be a situation in which plant fats with a high content of saturated fatty acids (e.g., coconut or palm fat) are used to produce hybrid products. It is therefore recommended that when creating new products that will be beneficial to the health of the population, manufacturers and researchers pay attention not only to the type and quality of the protein they contain but also the quality of fat.

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Correlation of machine learning-predicted viscosity of hydrocolloids with texture of plant-based meat analogue

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I. INTRODUCTION

Hydrocolloids have been extensively used in order to control the rheological properties of meat products due to their unique features. Therefore, if there is an efficient way to predict the rheological properties of hydrocolloids, it may be a breakthrough innovation in the meat processing industry. A machine learning framework was thus proposed in order to describe and predict the flow behaviors of six hydrocolloid solutions, and the predicted viscosities were correlated with the textural features of their corresponding plant-based meat analogues.

II. MATERIALS AND METHODS

The steady-shear viscosities of six food hydrocolloids (guar gum, locust bean gum, xanthan gum, konjac, methylcellulose, and sodium alginate) were experimentally measured at different levels of concentration, temperature, and shear rate using a controlled-stress rheometer. Their viscosity behaviors were then fitted into both mathematical and machine learning models, which were constructed in the Jupyter Notebook environment with the python programming language. The established machine learning model was also used to predict the viscosities of the hydrocolloids, which were correlated with the textural features of their corresponding plant-based meat analogues.

III. RESULTS AND DISCUSSION

Different shear-thinning and Newtonian behaviors were observed depending on the type of hydrocolloids and the shear rates. Methylcellulose exhibited an increasing viscosity pattern with increasing temperatures, compared to the other hydrocolloids (Figure 1(a)). The machine learning algorithms (random forest and multilayer perceptron models) in Figure 1(b) showed a better viscosity fitting performance than the constitutive equations (Power-law and Cross models).

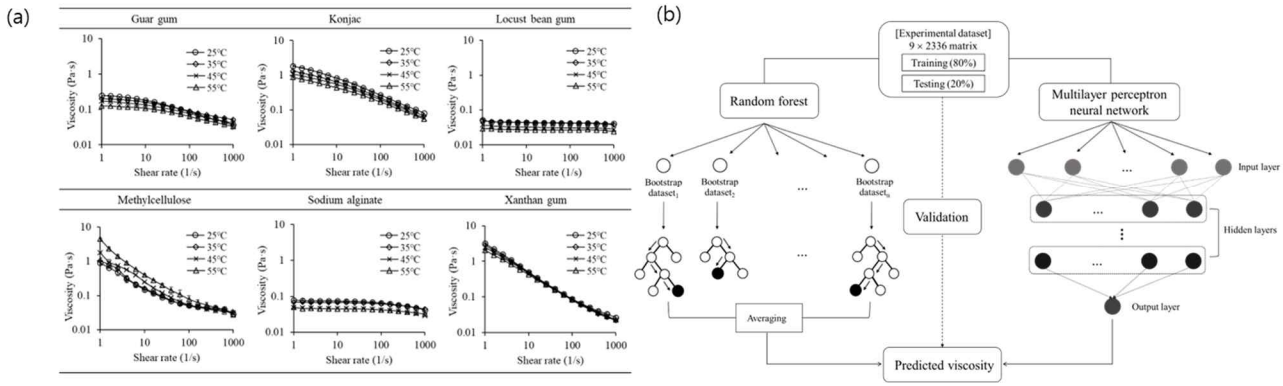

Figure 1. (a) Flow behaviors of hydrocolloids over shear rates and temperatures and (b) machine learning structure

Three hyperparameters of the multilayer perceptron model (optimizer, learning rate, and the number of hidden layers) were tuned using the Bayesian optimization algorithm, leading to the superior performance of the viscosity prediction (Figure 2).

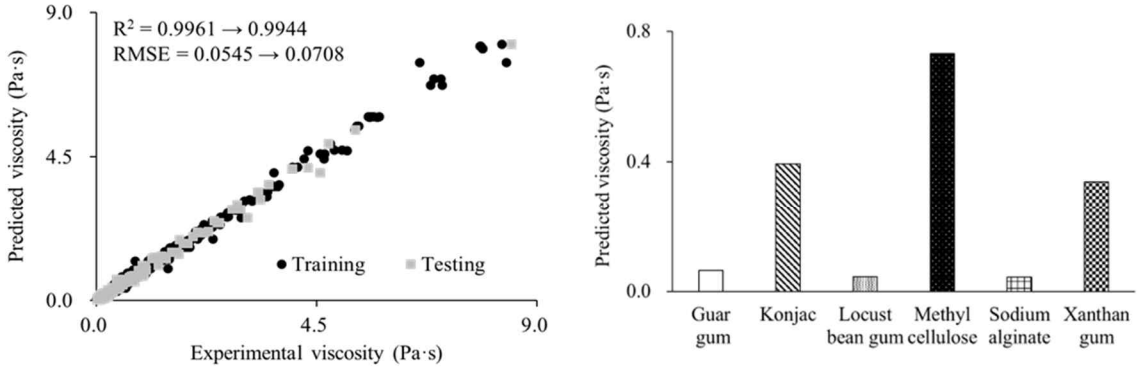

Figure 2. Machine learning predicted viscosity

The patty-making performance was highly dependent on the type of the hydrocolloids used. Methylcellulose was the most effective in preparing the patty samples without any cooking difficulties (Figure 2(a)). The predicted viscosities by machine learning showed similar patterns with the textural features of cooked meat analogues for all the hydrocolloids tested except for xanthan gum as shown in Figure 2(b).

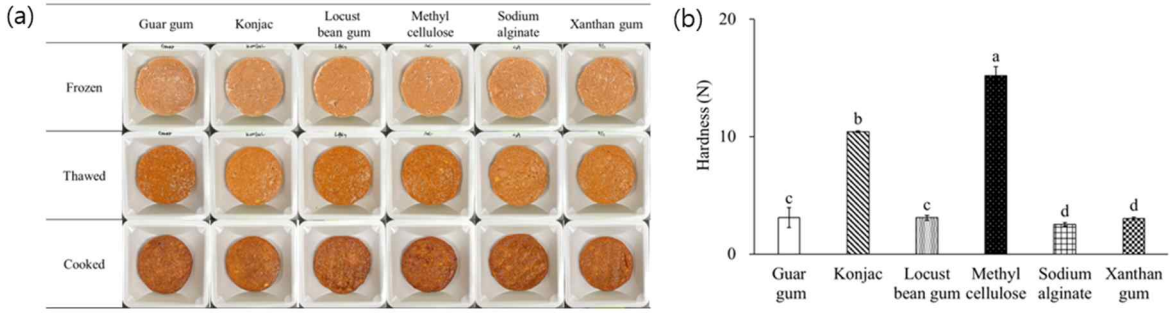

Figure 2. (a) Visual appearances and (b) textural properties of plant-based meat analogues

IV. CONCLUSION

In this study, machine learning might consequently be considered as an efficient tool to predict the rheological behaviors of hydrocolloid solutions under different processing conditions and further to estimate the textural properties of their corresponding end-products.

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INFLUENCE OF MEALWORM (*TENEBRIO MOLITOR*) FLOUR ADDITION AS MEAT REPLACER IN THE COMPOSITION AND MICROSTRUCTURE OF FRANKFURTERS WITH HEALTHIER LIPID CONTENT

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I. INTRODUCTION

Awareness of the importance of a healthy diet has increased, and the development of healthier meat products, mainly concerning their lipid content (quantity and quality), has gained attention [1]. In addition, there is a need for alternative and more sustainable sources of proteins to address the increasing protein demand caused by global population growth [2]. In this context, edible insects such as mealworms (*T. molitor*) may be an interesting choice for meat product reformulation because of their variety of nutrient compounds for human consumption, including proteins, healthier lipids (especially polyunsaturated fatty acids), minerals, and vitamins [3]. Some studies have already been conducted on partial meat replacement with mealworm in emulsified cooked sausages [4]. Therefore, this study investigated the composition and microstructure of meat replacement with mealworm flour (MF) in developing frankfurter sausages with healthier lipid content.

II. MATERIALS AND METHODS

Three different frankfurter treatments were manufactured [5]: a reduced fat control (RF-C) comprising 60% pork meat and 9% pork backfat and two treatments with MF addition as a meat replacer at 5% (MF5) and 7% (MF7). Sausages were properly homogenized, stuffed into cellulose casings, and heat-processed in a steam oven (CM-6, Rational, Germany) at 80 ºC for 60 min. After processing, the frankfurters were cooled overnight. The composition (moisture, fat, and protein), saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), and hardness (N) (TA.XT.plus, Stable Micro Systems, Surrey, UK) were analyzed. Microstructure was studied by a stereo microscope (Stemi SV6, Zeiss) and scanning electron microscopy (SEM) using a Jeol JSM-IT700HR (Jeol Ltd., Tokyo, Japan) field emission. Analysis of variance and Tukey's HSD test were done, and differences were significant when $p < 0.05$.

III. RESULTS AND DISCUSSION

The moisture content ranged from 64.06 to 68.66 g/100 g, with MF5 and MF7 treatments lower ($p < 0.05$) than RF-C. Protein and fat content were between 15.78 and 17.82 g/100 g and 11.69 and 15.20 g/100 g, respectively. Treatments with MF addition had higher ($p < 0.05$) protein and fat content than RF-C. Similar behavior was observed in hardness since the highest ($p < 0.05$) values were observed in frankfurters with 5% and 7% of MF (19.29 N and 18.74 N, respectively) compared with RF-C (14.09 N). The healthier profile of frankfurters was evidenced by higher MUFA and PUFA contents in MF7 compared with RF-C (64.09 and 14.09 and 59.85 and 12.19 mg/g, respectively). According to Figure 1, it was evident that the gel structure was well-formed in all treatments. However, small fragments of MF, probably in the form of chitin, were observed in the MF5 and MF7 treatments (Figure 1, top), which appear to act as filler particles and could be related to the highest ($p < 0.05$) hardness observed in these samples. The frankfurters exhibited a uniform, smooth, and complex structure in the SEM (Figure 1, bottom). Although some relevant cavities were observed in MF5 and MF7 samples (Figure 1, bottom), this was probably due to the chitin or other compounds of MF, which improves the gel

network strength (Figure 1, top), resulting in higher hardness. However, in the RF-C sample, those cavities seem smaller (Figure 1, bottom) but promote a higher destabilization of the gel network and lower hardness.

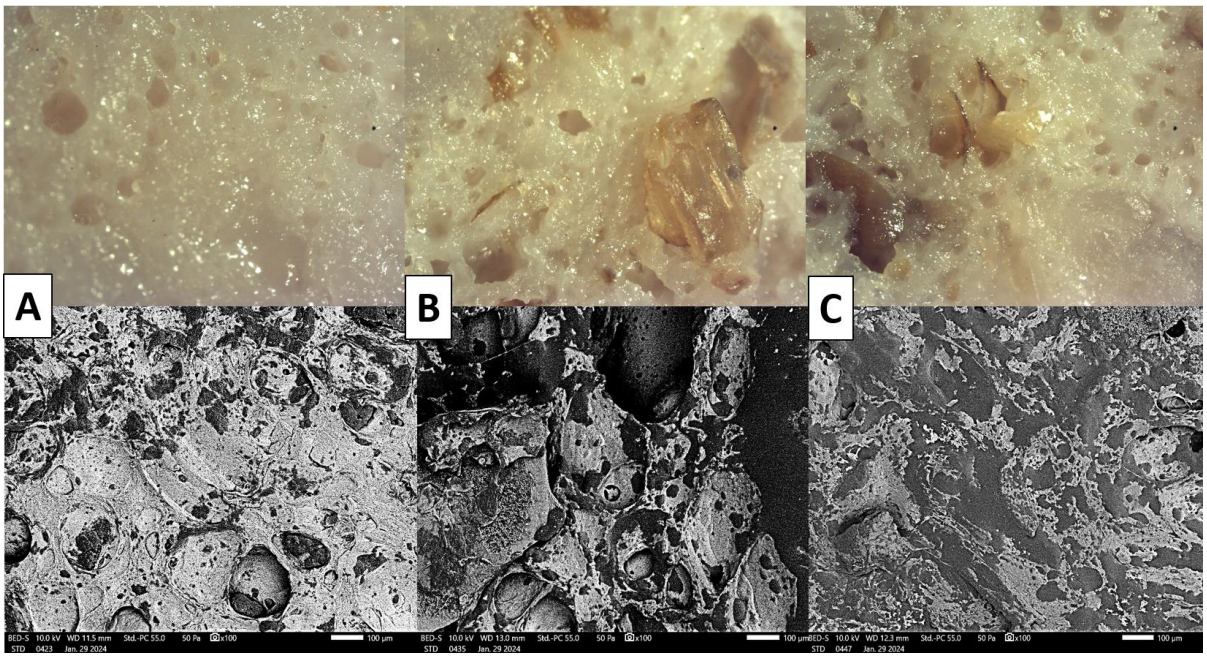

Figure 1. Microscopy photographs (top) and Scanning electron microscopy (bottom) of frankfurter sausages: A: RF-C = no MF addition; B: MF5 = 5% of MF addition; and C: MF7 = 7% of MF addition. Images at the top had a magnification of 4×. SEM images (bottom) white bar represents 100 µm.

IV. CONCLUSION

Incorporating MF at 5% and 7% levels affects the composition, textural, and morphological characteristics of frankfurter sausages in terms of improved protein and healthier lipid content, which were related to stronger hardness due to MF provides filler particles that reinforce the gel network of the products. In this respect, MF addition at 5 and 7% levels offers interesting potential to adapt the composition, texture, and structure of frankfurter sausages.

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POTENTIAL OF TAMARIND (*TAMARINDUS INDICA* L.) AS MEAT REPLACER IN FRANKFURTERS

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I. INTRODUCTION

Meat is highly nutritious, but some meat products, such as frankfurter sausages, particularly some of their components, are often considered unhealthy [1]. In response to the demand for healthier options, the concept of incorporating vegetables into meat products is gaining attention [2]. Brazil is recognized for its tropical fruit production, and the tamarind stands out. Thus, tamarind and its by-products in meat products are rich in healthy compounds such as vitamins and minerals, which could be an interesting option. Previously, some authors have used freeze-dried tamarind powder as a beef tenderizer [3]. Hence, the use of tamarind or its by-products can help develop healthier meat products and enhance their quality. Thus, the present study aimed to investigate the effect of different components from tamarind (pulp, seeds, peel) as meat replacers on the proximal composition, technological, and microbiology characteristics of frankfurters.

II. MATERIALS AND METHODS

To prepare the pulp, seeds, and peel from tamarind, the inner part of the fruit was soaked in water at a 1:1 (w/v) ratio and chilled storage for 24 hours. The pulp and seeds were manually separated, and then the seeds and peel were ground. Five types of frankfurters were prepared [4] replacing 5% of pork meat with 5% of tamarind components, resulting in the following treatments: a control without tamarind (T0), 5% of tamarind pulp (PT5), 5% of seeds (ST5), 5% of peel (CT5), and 5% of a mixture of them (PSCT5) as meat replacer. T0 was formulated with pork meat (60%), pork backfat (19), water (18.5%), and additives (2.5%), while in the rest of the samples, pork meat was replaced with 5% of each tamarind component. All ingredients were homogenized (Thermomix® TM6-1, Vorwerk Elektrowerke GmbH & Co., Germany), and the mixture was stuffed into cellulose casings (Viscase S. A., France) and heat-processed in a steam oven (Micro 40, Eller, Merano, Italy) at 80 °C and 99% RH for 60 minutes. After processing, the frankfurters were cooled until the next day, when the casings were removed. The proximate composition, processing loss (PL), pH, and microbiological analysis of frankfurters (Total viable count (TVC), Enterobacteriaceae, and mold and yeasts) were then performed. Analysis of variance and Tukey's HSD test were done, and differences were significant when $p < 0.05$.

III. RESULTS AND DISCUSSION

Using tamarind components as meat replacers significantly affected frankfurters' moisture, protein, fat, and ash content (Table 1). Moisture content ranged from 53.62 to 57.46 g/100 g. ST5 and PSCT5 did not significantly alter moisture content compared to T0 (Table 1). Regarding protein, T0 showed the highest ($p < 0.05$) content (Table 1) according to formulation since tamarind parts replaced pork meat, the primary protein source. Regarding fat, all samples with tamarind showed higher ($p < 0.05$) values than T0 (Table 1), probably due to the fat content of pulp, seed, and peel. Ash values of ST5, CT5 and PSCT5 were higher ($p < 0.05$) than T0, while PT5 showed similar ($p > 0.05$) values than T0. The moisture, protein, fat, and ash values in our study align with those reported by other studies on frankfurter [2]. The PL of frankfurters ranged from 6.02 to 16.46%. ST5 and PSCT5 showed lower ($p < 0.05$) PL than T0, while CT5 showed similar ($p > 0.05$) PL than T0. PT5 had the highest ($p < 0.05$)

PL. The pH values of frankfurters ranged from 6.16 to 6.41, with the lowest ($p < 0.05$) value in the PT5 and the highest ($p < 0.05$) in the T0 and ST5 treatments ($p < 0.05$) (Table 1). The lower pH is probably related to the acid pH of tamarind pulp (3.45), while the seeds and peel showed higher pH values (5.44 and 4.40, respectively). Regarding the TVC, values ranged from 1.65 log CFU/g (for the PSCT5 treatment) to 2.41 log CFU/g (for the CT5 treatment) ($p < 0.05$) (Table 1). The counts for Enterobacteriaceae and molds and yeasts were below the detection limits of the employed technique.

Table 1 – Proximal composition (g/100 g), processing loss (%), pH values, and microbiological analysis (log CFU/g) of frankfurters.

	Treatments				
	T0	PT5	ST5	CT5	PSCT5
Proximate composition					
Moisture	57.46 ± 0.22^{a}	55.74 ± 0.21^{b}	56.76 ± 0.44^{a}	53.62 ± 0.45^{c}	57.19 ± 0.30^{a}
Protein	16.23 ± 0.30^{a}	15.13 ± 0.34^{b}	13.75 ± 0.17^{c}	15.30 ± 0.36^{b}	14.03 ± 0.11^{c}
Fat	20.40 ± 0.61^{d}	24.75 ± 0.18^{a}	21.31 ± 0.12^{c}	23.36 ± 0.14^{b}	22.77 ± 0.35^{b}
Ash	2.44 ± 0.03^{c}	2.51 ± 0.01^{bc}	2.52 ± 0.04^{b}	2.59 ± 0.00^{a}	2.54 ± 0.01^{ab}
Processing loss	13.36 ± 0.71^{ab}	16.46 ± 2.82^{a}	6.02 ± 2.59^{d}	13.12 ± 0.70^{bc}	9.99 ± 1.77^{c}
pH	6.41 ± 0.02^{a}	6.16 ± 0.02^{c}	6.41 ± 0.01^{a}	6.20 ± 0.01^{b}	6.19 ± 0.01^{b}
Microbiological analysis					
TVC	1.84 ± 0.34^{ab}	2.34 ± 0.03^{ab}	1.83 ± 0.18^{ab}	2.41 ± 0.10^{a}	1.65 ± 0.07^{b}

Mean ± SD. Different superscript letters in rows (a-d) indicate statistically significant differences ($p < 0.05$).

IV. CONCLUSION

The study indicated that using different parts of tamarind (pulp, seeds, peel, or a combination) is a viable option to replace meat pork in frankfurters from a nutritional and microbiological point of view. Considering technological aspects, in terms of processing loss, using tamarind seed and peel as meat replacers in frankfurters resulted in the best options. This reformulation procedure improved the sustainable aspect of the production of frankfurters.

ACKNOWLEDGEMENTS

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Influence of muscle, freezing/thawing prior processing, and storage temperature on the formation of a white film on dry-cured meat

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I. INTRODUCTION

Drying during dry-cured meat production can lead to supersaturation of the residual water causing substances with low solubility to precipitate as crystals. The crystals can form within the muscle tissue influencing the texture or on the surface of dry-cured meat affecting its appearance [1]. Even if several studies on these crystals were conducted (e.g. [1-4]), the reason why and when precipitation primarily occurs on the surface or in the dry-cured meat is still unknown. Tyrosine is identified as one of the main components but also other aromatic amino acids as well as peptides and protein fragments have been identified [2]. Once formed, crystals can not necessarily be washed off permanently. Thus, ways to prevent crystallization must be found leading to the hypothesis that the muscle, freezing/thawing, and the storage temperature of dry-cured meat have a multifactorial influence on crystallization. Exemplarily the influence of loin and rump, freezing prior production (fresh *vs*. frozen and thawed), and the storage temperatures of 2 and 20 °C were investigated.

II. MATERIALS AND METHODS

Twelve pork loins and twelve pork rumps were purchased at 2 days postmortem. Six loins and six rumps were immediately frozen at -18 °C and thawed at day 4 p.m. at 20 °C. Other muscles were kept at 2 °C. At day 5 p.m. all muscle parts were salted with 3.6 % nitrite curing salt with 0.5 % $NaNO_2$, 0.7 % Tari Mix (ICL BK Giulini GmbH, Ladenburg, DE) and 0.02 % starter culture RPW consisting of *Staphylococcus xylosus* and *Staphylococcus carnosus* and *Debaryomyces hansenii* (AVO-Werke August Beisse GmbH, Belm, DE). Meat was stored at 2 °C and turned each third day. After 14 days, meat was rinsed with cold water and stored on a grid at 2 °C. After 3 days, grids were placed in a ripening chamber (KR 1 · 100 / E, Autotherm Ludwig Brümmendorf GmbH & Co KG, Waxweiler, DE) hold at 22 °C and 86 % relative humidity (rh) for 36 h, followed by 20 min smoking at 20 °C, and ripening for 24 h at 16 °C and 80 % rh. The parameters for drying were set at 15 °C and 75 % rh. Dry-cured meat was weighed weekly and pH was measured at d0, after salting (d22) and after ripening (d47) (testo 205, Testo, Lenzkirch, DE). Then, meat was vacuumized and stored at 2 °C. After an equalization period of 14 days, each loin and rump were cut in half and vacuumized. One half was stored at 2 °C and the other half at 20 °C. Appearance of crystals on the cut were monitored weekly. Statistical analysis of weight loss and pH was performed using the t-test and differences in the appearance of a white film were analyzed using Three-Way-ANOVA and Tukey's post-hoc test ($p < 0.05$) in SigmaPlot 15.0 (Systat Software Inc., San Jose, CA, USA).

III. RESULTS AND DISCUSSION

The pH-value of frozen/thawed loin is on d22 significantly ($p < 0.05$) higher than the pH of the fresh loin, whereas the pH of the fresh rump is significantly ($p < 0.05$) higher on d47 than of the frozen/thawed rump (Table 1). The weight loss is significantly ($p < 0.05$) higher for the frozen/thawed rump which is due to the destruction of cells by freezing [4]. However, no significant differences in the weight loss exist between fresh and frozen/thawed loin. These findings indicate that the muscle influences the weight loss during dry-cured meat production due to thawing, which was also illustrated by Bañón *et al.* [4], who showed significant different ($p < 0.05$) moisture contents in dependence of the muscle.

On the cut, only a white film of small crystals and not individual larger crystals have been identified starting with storage week 1 (Table 2). Contrary to Bañón *et al.* [4], who found more dry-cured meat with precipitates when meat was frozen and thawed in comparison to fresh meat, less dry-cured frozen/thawed

Table 1. pH-value and weight loss of dry-cured loin and rump with either fresh or frozen/thawed meat prior production.

	loin		rump	
pH ( )	**fresh**	**frozen/thawed**	**fresh**	**frozen/thawed**
d0	5.54 ± 0.06	5.59 ± 0.06	5.57 ± 0.04	5.59 ± 0.06
d22	5.44 ± 0.05	5.53 * ± 0.05	5.52 ± 0.07	5.49 ± 0.09
d47	5.51 ± 0.07	5.55 ± 0.04	5.62 ± 0.05	5.53 * ± 0.04
Weight loss (%)				
d0 to d22	6.05 ± 0.94	8.08 ± 1.01	2.95 ± 0.68	4.92 * ± 0.67
d0 to d30	30.75 ± 1.99	32.66 ± 1.90	21.45 ± 1.68	24.19 * ± 1.14
d0 to d47	46.04 ± 2.35	47.22 ± 1.99	32.28 ± 1.86	34.80 * ± 1.42

*indicate significant differences ($p < 0.05$) between fresh and frozen/thawed meat, determined using the t-test

loin and rump showed a white film than dry-cured meat made from fresh meat. This could be due to similar proteolysis indices of fresh and frozen/thawed meat [2]. Variations in the proteolysis between muscles [4] could have contributed to the significant different ($p = 0.012$) count of white films between dry-cured loin and rump in storage week 2, which was analyzed using Three-Way-ANOVA. Interestingly, in storage week 1 only 5 dry-cured rumps made of frozen/thawed meat, stored at 2 °C, showed a white film which vanished from week 1 to week 2 on four dry-cured rumps and was found again in week 3. Most of the other samples developed a white film from storage week 1 to week 2 or week 3. The white film on dry-cured meat stored at 20 °C vanished from week 5 to week 6 – except for 3 rumps made of fresh meat – indicating that a higher storage temperature leads to the white film being reduced. This is underlined by the Three-Way-ANOVA, which indicated significant differences ($p = 0.002$) between the storage temperatures in week 6.

Table 2. Count of appearance of a white film on the fresh cut surfaces of dry-cured loin and rump, from fresh or frozen and thawed meat. The dry-cured meat were stored at 2 °C or 20 °C (n = 6).

Muscle	**loin**				**rump**			
Prior processing	**fresh**		**frozen/thawed**		**fresh**		**frozen/thawed**	
Storage temperature	**20 °C**	**2 °C**	**20 °C**	**2 °C**	**20 °C**	**2 °C**	**20 °C**	**2 °C**
Storage week 1	0	0	0	0	0	0	0	5
Storage week 2	5	6	4	6	1	4	2	1
Storage week 3	6	6	6	6	6	6	5	5
Storage week 4	6	6	6	6	6	6	4	6
Storage week 5	6	6	6	5	6	6	4	6
Storage week 6	0	6	0	6	3	6	0	6

IV. CONCLUSION

A higher storage temperature (20 *vs*. 2 °C) of vacuumized dry-cured meat results in the disappearance of a white film on both, loin and rump, after six weeks of storage ($p = 0.002$). Moreover, more dry-cured loins showed a white film than did dry-cured rumps which could be due to the different muscle properties, e. g. muscle fiber sizes. However, short freezing prior production had no influence on the appearance of crystals on the cut, neither of dry-cured loin nor rump. As these findings are somewhat contrary to earlier studies, crystals contributing to the white film, should be characterized, the proteolysis index determined, the study extended and checked for differences in the crystallization in connection with the weight loss.

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HYBRID BURGERS BASED ON BEEF AND TEXTURIZED VEGETABLE PROTEINS

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I. INTRODUCTION

Meat and meat products are one of the most important sources of protein in the diet. The global population is growing, such that by 2050, it is estimated to exceed ~9 billion. While most of this expansion in population is expected to occur in developing countries, in high-income countries there are also predicted changes in demographics, with major increases in the number of older people, who have a greater requirement for protein. With a larger and older population, global needs for protein are set to increase [1]. The use of alternative sources of proteins that are not competing with meat production can be used to design hybrid meat products that can contribute to cover the new demand of protein. The combined use of meat and vegetable proteins is not something new. Since some decades ago, soya texturized protein has already been added in many meat products.

Another current problem is the loss of biodiversity due to the use of few crops (e.g., soya). There are some orphan crops that are been pushed to increase the biodiversity throw a European project (Cropdiva). One way to valorize these crops is to use texturized proteins obtained from these crops in hybrid meat products.

The aim of this study was to design and evaluate hybrid burgers by combining beef with different texturized vegetable proteins (TVP): soya (TEXPRO-M, Bankom Ltd) (S-TVP), lupin (Elementa Foods) (L-TVP), faba:pea (20:80, Trades) (FP-TVP) and pea (Nutralys T70S, Roquette) (P-TVP). An additional formulation with pea (P-TVP2) was used to study the use of a radish-based colorant (Shade Veggie Red, Exberry).

II. MATERIALS AND METHODS

Beef meat was minced and divided in five batches to prepare five formulations with similar contents of water (70%) and protein (18%), 50% of protein coming from TVPs, which were estimated from ingredients composition. The ingredients with common contents (g/kg) in the five batches were: breadcrumbs (37), salt (16), dextrose (1), white pepper (1), sodium sulfite (0.7), ascorbic acid (0.4), sodium ascorbate (0.3) and cochineal 4% (0.15). An additional batch was used to prepare a reference beef burger, with 880 g of beef, 60 g of water and the same formulation for the rest of ingredients. Table 1 shows the ingredients with different contents. The TVPs were previously hydrated with water in a water:TVP proportion of 2:1. The rest of water was added during the kneading of ingredients.

Table 1 – Ingredients with different addition to the different hybrid burgers (g/kg) and protein content of each TVP.

	Batch					
Ingredients (g/kg):	**Beef**	**S-TVP**	**L-TVP**	**PF-TVP**	**P-TVP**	**P-TVP2**
Beef	880	411	419	417	391	393
TVP	0	169	153	159	149	150
Water	60	363	371	367	403	385
Radish based colorant	0	0.8	0.8	0.8	1.6	16
TVP protein content (%)		51	56	54	65	65

Burgers were individually packed in a modified atmosphere (O_2/CO_2 gas mixture of 70/30) and stored at 4 ± 0.8°C exposed to 10 h lightness/14 h darkness cycles (LED tube T5 meat, PROMOLUX) with

an average intensity of 1000 lux. Visual appearance was evaluated just before packaging (day 0) and after 4 days of storage (before and after cooking). At day 4, burgers were unpacked and wrapped in aluminum foil. Then they were cooked in a pre-heated oven (SCC101, Rational) at 200 ºC until a core temperature of 69 ºC was achieved. Sensory analysis was carried out by 4 panelists, expert and trained on meat burgers according to ISO 8586:2023. Attributes and intensities were agreed-on the description of the hybrid burgers by consensus in two sessions. Attributes: characteristic non-meat odor and taste, sweetness, bitterness, springiness, crumbliness, juiciness and astringency.

III. RESULTS AND DISCUSSION

The raw hybrid burgers are more like a pork burger than a beef burger (Figure 1). The use of radish-based colorant at high dose gives the burger a more beef-like appearance after 4 days, but the color after cooking is too purple.

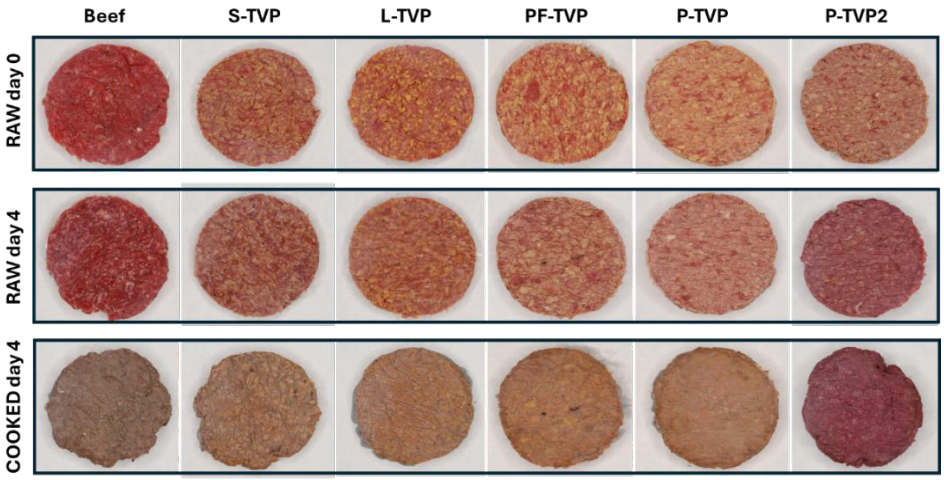

Figure 1. Burgers with different formulations: raw (days 0 and 4) and cooked (day 4)

TVP burgers had a specific smell and taste related to the plant origin of the added TVP (soya, lupin, fava or pea). In the FP-TVP, the fava flavor dominated over the pea flavor. Sensorially, P-TVP was the most neutral. All samples showed a satisfactory binding between the ingredients. In terms of texture, all TVP samples were scored with higher crumbliness, less springiness and similar juiciness than those of beef burgers. The S-TVP samples were slightly sweet and the L-TVP slightly bitter. All TVP samples had a final sensation of astringency but were not unpleasant.

IV. CONCLUSION

The hybrid burgers had a similar texture to the beef burgers, but each one had a specific odor and flavor (not unpleasant) according to the TVP added, being more neutral when the P-TVP was used. In terms of color, the TVP burgers looked more like a pork burger than a beef one. The radish-based colorant can be useful to confer a color more like beef, although the amount added must be adjusted and the color change produced by cooking must be considered.

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Analyses of the white film on fast produced dry-cured loin

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I. INTRODUCTION

During dry-cured ham production, substances with low solubility can precipitate, e. g. on the cut, as individual crystals or as a white film affecting the appearance [1]. Caused by the low water solubility, once formed, crystalized substances can not necessarily be washed off. In several studies the crystals have been analyzed, using e. g. gel chromatography [1], microscopy, electrophoresis [2], or HPLC [3]. These studies identified the amino acid tyrosine as one of the main components but also other aromatic amino acids, such as phenylalanine and methionine [2]. As the mentioned methods are targeted in terms of the substance to be analyzed, our aim is that the combination of the non-targeted methods digital microscopy (DigM), energy-dispersive X-ray (EDX), scanning electron microscope (SEM), and Raman spectroscopy (Raman) enables an open search for the identification of the substances precipitating on the cut.

II. MATERIALS AND METHODS

Two days p.m. 6 pork loins were frozen, thawed on day 4 p.m., salted on day 5 with 3.6 % nitrite curing salt with 0.5 % $NaNO_2$, 0.7 % Tari Mix (ICL BK Giulini GmbH, Ladenburg, DE) and 0.02 % starter culture RPW consisting of *Staphylococcus xylosus*, *Staphylococcus carnosus* and *Debaryomyces hansenii* (AVO-Werke August Beisse GmbH, Belm, DE) and stored at 2 °C. After 14 days, meat was rinsed with cold water, stored again at 2 °C on a grid and after 3 days placed in a ripening chamber (KR 1 · 100 / E, Autotherm Ludwig Brümmendorf GmbH & Co KG, Waxweiler, DE) hold at 22 °C and 86 % relative humidity (rh) for 36 h, followed by 20 min smoking at 20 °C, and ripening for 24 h at 16 °C and 80 % rh. Then, drying took place at 15 °C and 75 % rh. After 22 days of ripening, meat was vacuumized and stored at 2 °C. After 14 days, meat was cut in half to obtain a fresh cut and vacuumized again. The resulting surface was monitored weekly for the appearance of crystals. One loin was used to test the scheme for analyses of the white film and its crystals. The chosen loin (SELF) lost 44.7 % of its weight and had a pH of 5.52. To verify analyses, an industrial produced ham was analyzed in the same way and used as a control (CON). For analyses, a digital microscope (VHX-7000, Keyence, Osaka, JPN), an EDX with SEM (JSM 6460 LV, JEOL, Akishima, JPN), and a Raman (alpha300, WiTec, Ulm, DE) was used. Sample were analyzed with each method after the following steps: drying, washing with hexane, washing with water.

III. RESULTS AND DISCUSSION

Figure 1 presents the steps of analyses of the surface. The photo reveals differences in the density and distribution of the white film: white film of CON was distributed evenly on the whole surface, whereas white film on SELF was randomly distributed with a less white film in the middle of the loin. The less pronounced white film in the middle of SELF could be caused by a too short ripening time indicating an uneven weight loss, which could become even after additional storage time. With the DigM the crystals forming the white film became visible showing that the white film on both, SELF and CON, consists of small crystals with app. 0.1 mm in length. However, color and morphology of the crystal varies as crystals on SELF look like NaCl crystals, are transparent and rectangular with clear borders although crystals overlap. On the contrary, crystals on CON are whitish, dense, round with blurred borders, and do not overlap. These differences are also obvious in the SEM images, which clearly show differences in the morphology of the crystals. Crystals of CON are jaggy and show edges differentiating one from each other. Crystals on SELF also show clear edges, but crystals do overlap. Microscopy indicates that CON crystals might have grown next to each other, whereas SELF crystals do also grow on each other. However, even if the appearance of the crystals between CON and SELF vary, it is assumed that the crystals contain mostly one pure precipitated substance, which might differ between CON and SELF.

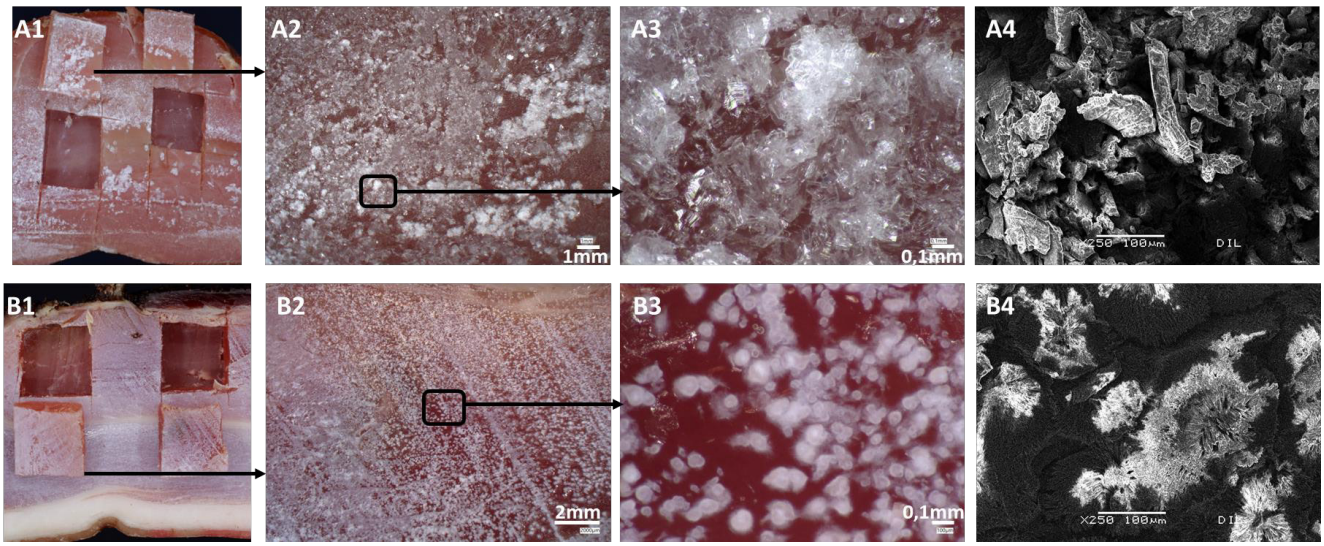

Figure 1. A: Self-produced dry-cured loin and B: control dry-cured ham, made form the leg: 1: photographic image, 2 and 3: digital microscopy with 20x and 200x magnification (the length of the bar indicates A2: 1 mm, B2: 2 mm, A3 and B3: 0,1 mm), and 4: scanning electron microscopy after washing with hexane (the length of the bar indicates 100 µm).

EDX of CON sample, which was dried and washed with hexane and water, had 10.13 ± 0.59 $\%_{mass}$ N, whereby SELF had 26.05 ± 2.67 $\%_{mass}$ N (n = 3). The N content in combination with the appearance of the crystalized substances, especially on CON, could lead to the conclusion of the presence of free, crystalized amino acids such as tyrosine [1, 2, 4]. Thus, Raman was used on CON and its database showed a probability of 76.55 to 87.92 % (n = 6) for *D*-tyrosine, which supports earlier studies [1, 3]. Since the Raman database of six positions of SELF indicated a high probability for different acids and oils, further analyses, such as X-ray diffractometry [4], and/or purification steps are needed to identify the substances.

IV. CONCLUSION

With the use of imaging methods, such as DigM, first insights of the white film on the cut of dry-cured meat can be gained. EDX in combination with SEM allows to check elemental composition and the morphology of the crystals on different positions to receive information about possible structures, such as if NaCl or amino acids are present. Despite the higher N content of SELF, Raman showed that tyrosine might not have been precipitate on the surface of SELF, but of CON. This indicates that further studies are needed to analyze dry-cured meat of 1) varying muscles, 2) different production processes, and 3) varying storage durations to check which factors influence the formation of crystals as a white film and what the receiving crystals are made of. To conclude, the aim was reached since the use of multiple non-targeted analytical methods enabled the identification of the white film made of small crystals on the cut of CON. However, as the identification of SELF crystals was not possible, the analytical procedure needs to be further adapted.

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EFFECT OF PORK HAM WITH PSE-LIKE QUALITY DEFECTS ON WET-CURED HAM

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I. INTRODUCTION

During the last decades, various quality defects have appeared in pork meat, specifically in ham muscles. The quality defects are typically characterized as low ultimate pH, abnormal color, increased drip-loss, reduced firmness, and disintegrated muscle fibers. Given their similarity to pale, soft and exudative (PSE) meat, they are often referred to as PSE-like quality defects. In 2018, the first reports regarding PSE-like quality defects in Norwegian ham were received. Similar defects have also been observed in several other European countries [1-3]. The observed quality defects cause significant financial losses for ham producers, particularly for cooked cured ham products [1]. Therefore, the aim of this study was to test and establish short-term solutions that make it possible to use raw materials with PSE-like quality defects. Different cooking times, drying, and various vacuum (press) were analyzed, and their effect on raw material with normal quality and PSE-like quality defects.

II. MATERIALS AND METHODS

Wet-cured ham was produced from ham cuts comprising the semimembranosus and adductor muscles. The control hams were of red, firm, and normal (RFN) meat quality, and the defect hams had PSE-like quality defects. Ham cuts were classified visually based on color, firmness and degree of disintegrated fibers. To include a diverse range of treatments and treatment combinations, a multifactorial design was applied. Both muscle types were injected with a 2.0 % NaCl and nitrite brine solution, giving a 20 % meat weight increase. The ham cuts for each of the cooked cured hams were tumbled in a vacuum drum (Rühle MKR 150, Rühle GmbH, Germany) with a cycle of 20 + 10 min for a total of 10 h. Following tumbling, half of the hams were dried for 30 min in an oven at 100°C. Meat cuts were then packed into vacuum bags, with loose or tight vacuum, to form wet-cured hams of approximately 0.5 kg each, where muscle types were mixed. The hams were cooked in water bath (Ico CKE 180, INGVALD Christensen A/S, Denmark) at 80°C for 2 h to obtain a core temperature of 73°C. Half of the hams were cooked for additional 45 min after obtaining a core temperature of 73°C. After cooking, the hams were cooled at -1°C for 2 h, then stored at 4°C for 4 days for maturation, prior to slicing. In total, there were 10 treatment groups, each with 3 replicates (Table 1).

Table 1 – Design with 10 treatment groups to study effect of raw ham quality on wet-cured ham.

Group ID	RFN ham (0) / PSE-like ham (1)	Drying after tumbling 0 min (0) / 30 min (1)	Vacuum under cooking Loose (0) / Tight (1)	Cooking time 2 h (0) / 2 h 45 min (1)	Number
A	1	1	0	0	3
B	1	1	0	1	3
C	1	1	1	0	3
D	1	1	1	1	3
CtrlB	0	1	0	1	3
E	1	0	0	0	3
F	1	0	0	1	3
G	1	0	1	0	3
H	1	0	1	1	3
CrtlG	0	0	1	0	3

After cooking and maturation, all hams were cross cut and sliced (Figure 1) and subjectively evaluated for visible cracks, muscle adhesiveness, texture, color and taste. Photos were taken of all cuts and slices.

III. RESULTS AND DISCUSSION

After salting, the adductor muscle from PSE-like meat was noticeable paler than the semimembranosus muscle, indicating lower absorption of the brine (Figure 2).
There was no indication for treatments to alleviate the PSE-like defect during curing and cooking. However, the small adductor muscle, which is located on the inside of the flat steak, particularly contributed to reduced quality of the cooked ham - it had remarkable looser texture with cracks, was dryer and had reduced taste (Figure 2). This reflects the lower absorption of the brine. The negative effect of the adductor muscle was also confirmed after a second round of producing wet-cured ham, where the two muscles were separated. Even though the semimembranosus muscle also had PSE-like zones, the adductor muscle with PSE-like zones had considerable additional negative effect on the final product – with increased occurrence of loose texture and dryness.

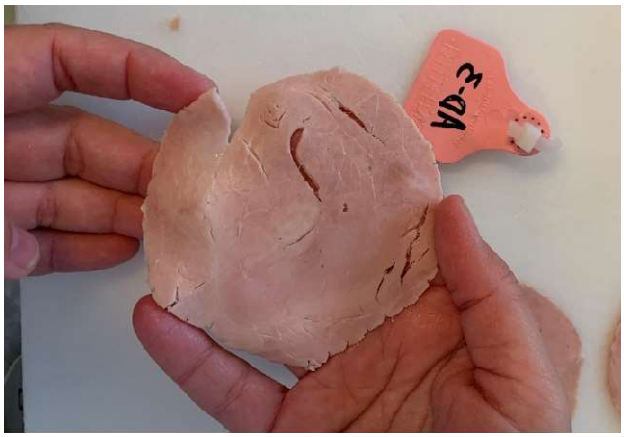

Figure 1. Slice of cooked ham with a slightly paler color than normal, and a loose structure with cracks.

Figure 2. Pale color of the *M.adductor* after tumbling indicates lower absorption of the brine.

IV. CONCLUSION

If the adductor muscle has signs of PSE-like quality defects, it has negative effect on the final quality in wet-cured hams. To reduce financial losses, it is proposed to remove afflicted zones before processing.

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ENHANCING STORAGE STABILITY AND QUALITY OF HYBRID BEEF-LENTIL SNACK BARS: THE ROLE OF STARCH GELATINIZATION AND LENTIL FLOUR ADDITION

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I. INTRODUCTION

Development of hybrid meat products created by integrating lentil into conventional meat products might address the growing demands for sustainable and healthier alternatives [1, 2]. Lentil has been associated with environmental and nutritional benefits, also provides potential functional properties, such as pasting and gelling, in meat products [3]. These benefits are attributed to the interaction between lentil carbohydrates with meat molecules, which might be influenced by starch gelatinization. This study explored the effect of addition level and starch gelatinization of infrared-heated lentil flour on the physicochemical properties and storage behavior of hybrid beef-lentil snack bars. Accelerated storage was utilized to simulate product deterioration during long term storage, which would be necessary for shelf-stable hybrid products to gain further acceptance for their convenience and long shelf life [4].

II. MATERIALS AND METHODS

Lentil flour with higher starch gelatinization (27% damaged starch/g flour, db) was obtained by tempering green lentil seeds, initially containing 10% moisture, to a target moisture level of 25% for 24 h. Subsequently, both the tempered (HG) and non-tempered (NT) seeds underwent infrared heating to a surface temperature of 150°C then were milled into flours. The beef-lentil snack bars were produced using procedures modified from jerky production [5]. Beef outside round (*Biceps femoris*) was trimmed and ground through a 9.5 mm plate. An all-beef formulation was prepared (Con). Flours were substituted for beef at 6, 12, 18% w/w (NT6, NT12, NT18, HG6, HG12). The meat mixture with HG flour at 18% addition was too thick to stuff and dropped from further testing. Other ingredients included brown sugar, salt, soy sauce, glucono-delta-lactone, encapsulated citric acid, sodium erythorbate, sodium nitrite, and spices. Mixtures were stuffed out as bar strips and then cooked and dried to a target water activity of 0.90. Samples of all treatments reached an internal temperature of 72 °C. Dried strips were equilibrated for seven days, then individually vacuum packaged in jerky pouches (thickness 3 µm, oxygen permeability < 60.0 cc / m2 / 24 h). Two storage conditions were employed: room temperature (RT), approximate 20 °C, 25% relative humidity (RH), and accelerated storage (HT), approximate 40 °C, 25% RH, for up to 90 days. Three replications of all treatments were prepared, and results were analyzed by a linear mixed model using R.

III. RESULTS AND DISCUSSION

Samples of all treatments reached a pH below 5.3 and water activity under 0.90, complying with Canadian regulations for shelf-stability. Cook yields were approximate 63%. As flour addition level increased, carbohydrate content of beef-lentil bars increased (14.5 to 28.1%), fat content decreased (8.3 to 4.3%), but protein content remained constant (around 33%). Beef-lentil bars effectively retained moisture under RT storage, while Con, the beef only treatment, showed significantly ($p<0.05$) lower moisture content (36.1 to 25.9%) after 90 d. Under accelerated HT storage, HG12 and NT18 bars maintained similar moisture levels after 90 d, while other treatments (Con, NT6, HG6, NT12) experienced significant moisture loss. This loss likely led to unfavorable physicochemical changes,

also suggesting that the standard jerky pouch may not provide sufficient barrier properties for long-term storage. Samples of HG12 and NT18 displayed a lighter (higher *L**) and more yellow (higher *b**) color than Con. Samples of all treatments became darker, less red, and less yellow in color during storage, while those with increased lentil flour addition and starch gelatinization showed less change in color (more stable color over time). Product stability was defined here as maintaining similar physicochemical properties to day 1. Interestingly, Con exhibited the most consistent and stable *L**. Beef-lentil bars under accelerated HT storage had greater reduction in sample redness (*a**) than Con, as storage time increased. Beef-lentil bars with increased addition of lentil flours and greater starch gelatinization had higher WB shear values initially, also showing less change during storage than Con. Using a three-point bending test, the break force of the strips increased as flour addition level increased, but were not affected by starch gelatinization level. Only NT18 showed a significantly higher stiffness (defined as break force / deformation) than Con, a common indicator of cohesiveness of non-meat snack bars. Beef-lentil bars under accelerated HT storage showed greater increase in stiffness as storage time increase. NT18 also exhibited lower lipid oxidation level, as measured by thiobarbituric acid reactive substances (TBARS), on day 1, highlighting the potential benefit of lentil flour in inhibiting oxidation. NT18 consistently maintained lower lipid oxidation levels throughout 90 d of accelerated HT storage. HG12 also showed lower incremental increase in TBARS values than Con after 60 d. This aligns with earlier findings on the antioxidant properties of infrared-heated lentil flours and fractions in meat processing [3].

IV. CONCLUSIONS

This study demonstrates the significant benefits of integrating lentil flour into shelf-stable meat products, particularly in enhancing moisture retention, color stability, and reducing lipid oxidation under accelerated high temperature storage. Starch gelatinization played an important role in these improvements, suggesting its potential to maintain product quality in long-term and more extreme storage. However, significant time by condition interaction effects were identified, pointing to the need for more accurate storage simulations that better represent typical consumer conditions. Additionally, the detailed interactions between partially gelatinized lentil starch and meat molecules in dried meat systems are not fully understood. Further microscopic analysis could provide deeper insights into these molecular dynamics. There is also a strong recommendation for future products to incorporate better packaging solutions and ingredient selection to extend shelf life and maintain product integrity. These results underscore the promise of lentil ingredients as sustainable and functional ingredients in meat products, meriting further research to optimize hybrid product formulations.

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SENSORY CHARACTERISTICS OF CANNED CORNED BEEF CURED WITH CELERY EXTRACT

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I. INTRODUCTION

Corned Beef is the industrialized meat product, produced exclusively with beef, cured, cooked, hermetically packaged, commercially sterilized and quickly cooled [1]. The typical curing of the product is made with synthetic sodium nitrate and nitrite, providing preservation, typical color and the control of the lipid oxidation. Corned Beef can also be made with other curing agents as long as the food safety and the commercial requirements of the final product is not compromised [1]. As a way to meet the demand of current consumers toward natural foods, the meat industry has been searching for alternative sources of nitrates and nitrites to replace the traditional synthetic curing salts. Celery extract is naturally rich in nitrate (which can be reduced to nitrite) and a candidate to replace the synthetic salts in the preparation of cured meat, thus allowing the claim of natural curing. To achieve this, it is important that the final product presents sensory characteristics similar to those of the original product [2], as they are the first ones to be considered by the consumer. The present study aimed to analyze the sensory characteristics and the acceptance of Canned Corned Beef (CCB) made with celery plant extract to replace sodium nitrite.

II. MATERIALS AND METHODS

The following CCB formulations were prepared: F1) standard formulation (control), containing 0.01% sodium nitrite, 1.1% sucrose and 2.3% NaCl; F2) 0.12% celery extract, 1.1% sucrose, 1.3% NaCl and 0.7% BR flavouring; F3) 0.12% celery extract, 1.1% sucrose, 1.3% NaCl and 0.7% UK flavouring; F4) 0.12% celery extract, 1.1% sucrose and 1.3% NaCl and F5) 0.12% celery extract, 1.1% sucrose and 2.3% NaCl. The celery extract used was ACCEL™ XP30 (Kerry Inc.; 30,000 - 36,000 ppm $NaNO_3$ equivalent). The formulations were hermetically canned (340 g per can) and commercially sterilized according to the Brazilian legislation [1]. The residual sodium nitrite in the formulations was determined according to AOAC [3].

The sensory analyses were performed according to Brazilian standards [4] with 45 untrained panelists, who manifested their awareness and agreement in a Free and Informed Consent Form (CEP-FOA/Unesp number 4.561.244). The formulated CCB were offered to the tasters at 7 ºC on white plastic plates as 1 cm^3 cubes coded with three random digits. For the multiple comparison test, the control was first set as a reference to the attributes “salty taste”, “meat flavor”, “after taste” and “color”. Then the panelists were invited to evaluate how intense each of the sensory characteristic was in relation to the control, using a 5-point multiple comparison scale, in which 1 corresponded to “extremely less intense than control” and 5 corresponded to “extremely more intense than control”. For the acceptance test, the panelists tasted one sample of each formulation and evaluated the attributes “meat flavor”, “salty taste” and “general impression” according to their preference, using a 5-point hedonic scale in which 1 corresponded to “I dislike it very much” and 5 to “I like it very much”.One-way ANOVA was used for the statistical analysis and Duncan's test was used to compare different means at 5% of probability.

III. RESULTS AND DISCUSSION

Except to F4, all the formulations made with celery extract were considered equivalent to the standard product made with sodium nitrite regarding to the sensory attributes evaluated (Fig. 1). Due to the lower content of NaCl, F4 differed from the other formulations in “salty taste” and “meat

flavor", showing itself to be the least favorable to replace nitrite curing in CCB.The residual nitrite concentrations in the formulations were (ppm): F1 8.23; F2 6.79; F3 7.62; F4 6.49; F5 6.51, thus meeting the requirements of the Brazilian legislation (maximum 150 ppm; [5]).

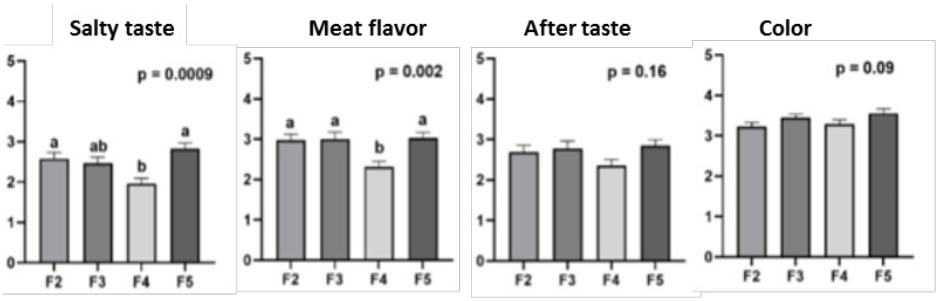

Figure 1. Multiple comparison test carried out with the experimental Canned Corned Beef.

All the formulations received equivalent scores in the acceptance test for "meat flavor", "salty taste" and "general impression" (Table 1), thus confirming the suitability of the celery extract to provide the characteristics of CCB. So we can say that the products made with celery extract had the same acceptability as the product made with synthetic sodium nitrite. In addition, celery extract is highly compatible with processed meat products, due to its low pigmentation and mild flavor, which do not interfere with the color and the taste of the final product [6]. These results confirm the use of celery extract to produce naturally cured CCB, without any disadvantage to its organoleptic characteristics.

Table 1 – Acceptance test of the experimental Canned Corned Beef.

Attributes	Formulations					p value
	F1	F2	F3	F4	F5	
Meat flavor	3.44 ± 0.121	3.38 ± 0.143	3.38 ± 0.147	3.22 ± 0.149	3.47 ± 0.144	0.76
Salty taste	3.30 ± 0.151	3.24 ± 0.135	3.22 ± 0.155	3.11 ± 0.163	3.53 ± 0.141	0.36
Overall impression	3.20 ± 0.129	3.16 ± 0.149	3.29 ± 0.148	3.16 ± 0.162	3.42 ± 0.140	0.66

IV CONCLUSION

The celery extract used as the agent of natural cure to produce Canned Corned Beef was able to provide the original characteristics of the synthetic nitrite cured product and reached the consumers acceptance.

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EFFECT OF HIGH HYDROSTATIC PRESSURE COMBINED WITH SODIUM CHLORIDE AND SODIUM PHOSPHATE ON THE PALATABILITY OF CHICKEN MEAT GELS

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I. INTRODUCTION

The growing number of older adults, including those with chewing and swallowing disorders (dysphagia), creates a high market demand for texture-modified food products for hospitals, nursing homes, and home consumption [1]. Often, especially among older people with dysphagia, malnutrition is a concern, and protein-rich foods are usually needed to improve their nutritional status. Additionally, a wide variety of foods with different aromas and flavors can help stimulate appetite in this population [2,3]. Various food processing technologies, such as high hydrostatic pressure processing, high hydrodynamic pressure processing, pulsed electric field treatment, plasma processing, ultrasound-assisted processing, and irradiation, are used to modify the texture and sensory characteristics of foods while maintaining their nutritional value [4]. The objective of this study was to investigate the effect of high hydrostatic pressure (HHP) at 0.1-200 MPa for 10 minutes at 20°C in combination with sodium chloride and sodium phosphate addition on the sensory evaluation and mastication parameters of chicken meat gels.

II. MATERIALS AND METHODS

The chicken breast meat was used for meat gel preparation, following the method of Maksimenko et al. [5]. Briefly, the minced chicken meat was mixed with various concentrations of sodium chloride (0-2%) and sodium pyrophosphate (0-0.3%) and subjected to HHP treatment at 0.1-200 MPa for 10 min using a high pressure food processor (Dr. CHEF, Kobe Steel, Japan), followed by heat treatment at 80°C for 30 min. The sensory evaluation of chicken meat gels was conducted using Scheffe's paired comparison method. Twenty-one untrained panelists (10 males and 11 females; aged 21-31 years) from the Faculty of Agriculture at Niigata University of Japan were selected based on their interest and availability to participate in the study, in accordance with ethical standards. Participants were asked to assess the odor, taste, texture, and overall acceptance of thermal meat gels on a 7-point scale ranging from −3 to +3. Friedman's test was used to compare significant differences between the scores for each evaluation item at the 5% level. For the masticatory test, ten untrained panelists (5 males and 5 females; aged 21-31 years) from the Faculty of Agriculture at Niigata University of Japan were selected based on their interest and availability to participate, in accordance with ethical standards. Each participant's total chewing time duration (s) and the number of chewing cycles (n) before the first swallow were determined. A confidence level of 1% was used to compare significant differences among means using the Student's t-test.

III. RESULTS AND DISCUSSION

Food textures recommended for dysphagia diets (patients with chewing and swallowing dysfunctions) should be soft, moist, elastic, smooth, and easy to swallow [6,7]. Our previous studies showed that pressurized meat gels at 150-200 MPa exhibited higher hardness, cohesiveness, and elasticity compared to unpressurized meat gels, according to the texture profile analysis [5]. In this study, the

sensory evaluation of the pressurized chicken meat gels at 150-200 MPa showed high scores for the "Hardness," "Juiciness," "Cohesiveness," "Springiness," "Easy to swallow," and "Pleasant taste" items ($P < 0.05$) compared to unpressurized chicken meat samples, irrespective of the sodium chloride and sodium phosphate content. The panelists reported that the texture of pressurized meat gels was moist, elastic, more pleasant to bite, and easy to swallow. In the mastication experiment, the number of chewing cycles (n) and chewing time duration (s) increased in pressurized meat gels at 150-200 MPa compared to unpressurized meat samples ($P < 0.01$).

IV. CONCLUSION

HHP treatment at 150-200 MPa was effective in providing high cohesiveness and elasticity; however, it resulted in low softness. The increased hardness may have affected the number of chewing cycles and chewing time in the mastication test. Nevertheless, panelists reported that the texture of pressurized meat gels was more pleasant to chew and easier to swallow due to the increased moisture and elasticity of the meat bolus. Future research on the analysis of bolus formation and transportation is needed, as it is an important aspect of texture and swallowing characteristics.

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THE ROLE OF POTASSIUM AND CALCIUM CHLORIDE IN TECHNOLOGICAL AND SENSORY PROPERTIES OF SODIUM-REDUCED HYBRID BOLOGNA SAUSAGES

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I. INTRODUCTION

The growing consumer demand for healthier and more sustainable food options has stimulated research into developing meat alternatives, including hybrid meat products, which are partially replaced by more sustainable protein sources, primarily plant proteins. Although interest in hybrid meat products has increased in recent years, only a limited amount of research has evaluated the impact of replacing sodium chloride with potassium and/or calcium chloride in these products. Therefore, this study investigates the reduction of NaCl in soy-based hybrid bologna sausage, specifically focusing on its replacement with KCl and $CaCl_2$.

II. MATERIALS AND METHODS

Six treatments were elaborated in three independent batches, as displayed in Table 1. The formulations were prepared following a previous methodology [1]. Emulsion stability, water activity (Aw), and pH were measured at room temperature (24-25 °C) in nine replicates according to methodologies described by Paglarini et al. 2019 [1] . Texture was evaluated based on a previous methodology [2] using 18 samples from each treatment. Sensory tests received approval from the Ethics in Research Committee under CAAE number 31660120.0.0000.5404. Sensory acceptance and the Check All That Apply (CATA) test were conducted with 112 consumers according to a previous methodology [3]. Differences between treatments were evaluated using one-way ANOVA and the post-hoc Tukey's test with 95% confidence in SPSS software.

Table 1 – Formulations (g/100g) of sodium-reduced hybrid Bologna sausages

Ingredients	Treatments					
	C	HC	H-NaCl	H-KCl	H-$CaCl_2$	H-KCl/$CaCl_2$
Beef	65.00	32.50	32.50	32.50	32.50	32.50
Hydrated soy protein*	-	32.50	32.50	32.50	32.50	32.50
NaCl	2.00	2.00	1.00	1.00	1.00	1.00
KCl	-	-	-	1.275	-	0.6375
$CaCl_2$	-	-	-	-	0.6264	0.3132
Water	12.04	12.04	13.04	11.76	12.41	12.09
Ionic Strength	0.3422	0.1711	0.3422	0.3422	0.3422	0.3422

All treatments were elaborated with 10% pork back fat and 10% canola oil, 0.015% nitrite, 0.05% sodium erythorbate, 1.5% Bologna sausage condiment, and 0.003% carmine dye. * 20% of crude protein content.

III. RESULTS AND DISCUSSION

The emulsion stability (Figure 1A), pH, Aw, springiness, and cohesiveness (Table 2) were higher in hybrid bologna sausages compared to the traditional one (C), except for the treatments containing $CaCl_2$. This salt disrupted the protein matrix by reducing protein solubility, affecting emulsification and

gelation properties. The results can likely be attributed to the effect of divalent ions on protein chains and the pH reduction caused by $CaCl_2$. The control treatment exhibited greater hardness, which was expected since soy does not have the same gelling behavior as meat. Both hybrid bologna sausages, with either 100% NaCl or 50% NaCl and the addition of KCl, demonstrated high sensory acceptability.

Table 2 – Physicochemical results of sodium-reduced hybrid Bologna sausages

Parameters	Tratamentos						SEM
	C	HC	H-NaCl	H-KCl	H-$CaCl_2$	H-KCl/$CaCl_2$	
pH	5.95^{D}	6.30^{B}	6.34^{A}	6.33^{A}	5.76^{E}	6.08^{C}	0.03
Aw	0.9744^{F}	0.9782^{D}	0.9862^{A}	0.9776^{E}	0.9830^{B}	0.9801^{C}	0.001
Hardness (N)	44.91^{A}	33.65^{B}	27.62^{D}	30.74^{C}	23.37^{E}	25.47^{E}	0.88
Springiness (mm)	0.861^{B}	0.883^{A}	0.878^{A}	0.890^{A}	0.858^{B}	0.870^{B}	0.002
Cohesiveness	0.569^{B}	0.691^{A}	0.598^{B}	0.669^{A}	0.423^{D}	0.481^{C}	0.013

Treatments with the same letters in the lines did not differ according to the Tukey's test. SEM: Standard error of mean.

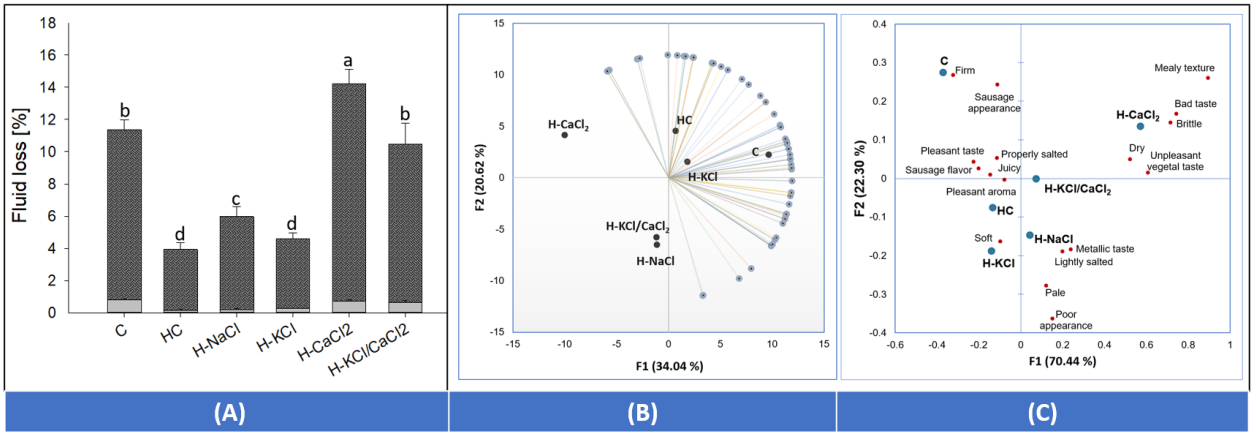

Figure 1. (A) Fluid loss (water in black and dry matter in grey), (B) internal preference mapping of overall acceptance and (C) CATA results of sodium-reduced hybrid Bologna sausage treatments. Treatments with the same letter in the columns did not differ from each other according to Tukey's test.

IV. CONCLUSION

We concluded that the impact of salt reduction in hybrid bologna sausages was partially minimized by the soy protein. Overall, the partial substitution of NaCl with KCl is feasible considering technological and sensory aspects hybrid emulsifying meat products. $CaCl_2$, on the other hand, is not suitable for reducing NaCl in soy-based hybrid Bologna sausages.

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VALORIZATION OF MANILA TAMARIND PEEL (*PITHECELLOBIUM DULCE*) AS A MEAT PRODUCT ADDITIVE

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I. INTRODUCTION

The meat industry repeatedly explores natural alternatives to improve the quality and stability of meat products. Food additives play a determining role here since they provide physicochemical, technofunctional, and preservative characteristics. These substances are specially added to foods to improve their characteristics throughout production, processing, and storage [1,2]. The manila tamarind (*Pithecellobium dulce*) is a tree in the Fabaceae family native to Mexico and Central America. This tree produces curved, twisted pods with black seeds surrounded by edible white, pink, or reddish pulp. The pulp has a sweet and slightly sour taste and is often eaten fresh as a seasonal fruit. In addition to its culinary uses, the pods and other parts of the manila tamarind tree are also used in traditional medicine [3]. The aim of this study was to investigate the potential of powders extracted from manila tamarind peel as a natural additive for meat products to improve their quality and safety.

II. MATERIALS AND METHODS

The manila tamarind peel was collected from plants located in the municipality of Concordia Sinaloa, Mexico. The plant material was sanitized, dried at room temperature (35°C), and pulverized in an electric grain mill (20 mesh particle size). The powder was subjected to physicochemical (pH and Hex color) and technofunctional characteristics (WHC water retention capacity, OHC oil retention capacity, SWC swelling capacity, and GFC gel formation capacity); texturized soy was used as a commercial standard. Additionally, in vitro, TTC-tannins, TPHC-total phenolic, and CGA-chlorogenic acid contents, as well as FRSA-free radical scavenging activity and FRAP-ferric reducing antioxidant power, were evaluated in an aqueous extract obtained from manila tamarind (MTAE). MTAE was incorporated in a pork meat system (incubated at 65 °C/1 h) and evaluated for lipid oxidation through the TBARS-thiobarbituric acid reactive substances assay. Butylhydroxytoluene (BHT) was used as a positive control [4]. Obtained data (n=6) were subjected to t-tests at $P<0.05$ (NCSSv11).

III. RESULTS AND DISCUSSION

Table 1 shows that manila tamarind powder showed lower pH values than texturized soy ($P<0.05$). Hex color codes indicate that manila tamarind and texturized soy powder color names were Pale Taupe and Tan, respectively. Concerning technofunctional properties, manila tamarind powder showed the highest WHC and OHC values, while texturized soy showed the highest ($P<0.05$) SWC and GFC.

Table 1 – Physicochemical and technofunctional characterization of manila tamarind powder.

Item	pH	HEX Color	WHC	OHC	SWC	GFC
Manila tamarind	5.80±0.01	#c3a390	78.60±0.55	59.90±0.50	56.00±1.01	N.D.
Texturized soy	6.48±0.01	#d7c4a1	70.15±1.10	48.01±0.90	84.80±0.65	57.19±0.30

P-value	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2 shows the presence of polyphenol compounds (TTC, TPHC, and CGA) in the aqueous extract of manila tamarind powder. Concerning antioxidant assays, non-differences were found in TTC and TPHC of Pleurotus powders, while TFC was not detected (N.D.) (P>0.05). Also, the highest FRSA and FRAP values were displayed by BHT (P<0.05). Furthermore, pork meat with manila tamarind aqueous extract exhibited the lowest (P < 0.05) lipid oxidation levels.

Table 2 – Polyphenol content, antioxidant activity, and lipid oxidation inhibition of MTAE.

Item	TTC	TPHC	CGA	FRSA	FRAP	TBARS
Manila tamarind	67.00±3.54	27.70±0.90	100.20±3.22	43.03±1.10	0.50±0.05	0.27±0.01
BHT	-	-	-	79.90±0.61	1.99±0.05	0.40±0.02
P-value				<0.05	<0.05	<0.05

According to the current results, powders from natural sources with pH values close to neutrality and flours in low concentrations may not affect their physicochemical and technofunctional characteristics [5,6]. Also, phenolic compounds in manila tamarind samples have been demonstrated to be associated with their bioactivity [1]. Also, it has been reported that phenolic compounds from natural products reduce lipid oxidation in meat and meat products [6]. However, the preservative effect on food systems of manila tamarind, has not been reported.

IV. CONCLUSION

Physicochemical results indicate that manila tamarind powder showed pH values near neutrality and a Pale Taupe color. The results evidence that the evaluated powder exerts technofunctional properties, including WHC, OHC, and SWC. The presence of polyphenol compounds and their antioxidant activity were also demonstrated. Additionally, MTAE showed the highest effect against lipid oxidation in a meat system. Based on the above, manila tamarind powder can be proposed as a natural additive for meat products.

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THE INFLUENCE OF PACKAGING TYPE AND ANTIOXIDANTS (NATURAL VS. SYNTHETIC) ON THE TEXTURE AND COOKING PROPERTIES OF CHICKEN BURGER

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I. INTRODUCTION

Chicken burgers are popular and affordable products, but their high unsaturated lipid content makes them prone to lipid oxidation. To control this process, vacuum packaging and antioxidant additives are commonly used, extending the product's shelf life. However, with increasing demand for clean-label products, synthetic antioxidants are being replaced by natural substances with antioxidant properties. Pink pepper (*Schinus terebinthifolius* Raddi) has been recognized for its bioactive compounds and antioxidant activity [1]. Our previous findings [2] showed that pink pepper extract has antioxidant potential to delay lipid oxidation in chicken burgers. To complement this work, this study aimed to assess the influence of packaging and antioxidants (pink pepper extract and butylhydroxytoluene - BHT) on chicken burgers' texture and cooking properties for 7 days at 4 °C.

II. MATERIALS AND METHODS

Pink pepper extract (PPE) was prepared according to the described by Menegali et al. [2]. Three burger formulations were produced [2]: a control without antioxidants (C), one with the synthetic antioxidant BHT (90 mg BHT/kg meat), and one with natural antioxidant (PPE) (volume of PPE equivalent to 90 mg gallic acid equivalent/kg meat). These three samples were packaged in both aerobic (A) and vacuum (V) packaging, resulting in six treatments. Burgers were evaluated in triplicate after 1 and 7 days at 4 °C for texture profile analysis (TPA) and cooking properties (cooking loss and diameter reduction). Additionally, their composition (moisture, fat, protein, and ash) was analyzed [3]. Proximate composition data were analyzed considering treatments as a fixed effect and replicates as a random effect. Texture profile analysis data were analyzed by a factorial design with fixed effects as treatment (3), packaging (2) and storage time (2), and their interaction. Results were evaluated by ANOVA followed by Tukey's test ($p<0.05$).

III. RESULTS AND DISCUSSION

No effect of antioxidant was found on moisture and protein. Only lipid and ash contents were significantly affected by the treatments, showing marginal differences among samples (Table 1). Regarding TPA, pink pepper extract significantly reduced springiness (PPE: 0.78) compared to the control (0.82) and BHT (0.81) samples, but had no effect on other TPA parameters. All texture parameters showed a significant interaction ($p<0.05$) between packaging and storage time. After 7 days of refrigeration, samples had higher hardness, cohesiveness, and chewiness than the initial storage period. The possible occurrence of protein oxidation during storage, which could result in protein cross-linking, may have impacted the structure of muscle protein, increasing hardness [4]. Among these samples, the vacuum-packaged ones were significantly harder and more cohesive than those packaged aerobically (Figure 1), which could be attributed to increased exudation in vacuum-

packaging. Similarly, cooking loss increased after 7 days of refrigeration, with vacuum-packaged samples experiencing the most pronounced effect. This could also be related to exudation. Only storage time significantly impacted diameter reduction, with samples after 7 days of refrigeration showing higher reductions after cooking (13.86%) compared to freshly processed samples (10.90%). Diameter reduction occurs due to meat protein denaturation with water and fat loss. Therefore, it was expected that the burgers would have a smaller diameter after 7 days of refrigeration because of greater cooking loss. Lim and Rosli [5] also reported shrinkage in beef burgers during storage.

Table 1 – Proximate composition (g/100g) of raw chicken burgers

Samples	Moisture	Protein	Fat	Ash
C_A	73.70 ± 0.62 ^{a}	13.86 ± 0.30 ^{a}	4.19 ± 0.05 ^{c}	2.29 ± 0.07 ^{ab}
BHT_A	73.06 ± 1.14 ^{a}	13.45 ± 0.95 ^{a}	4.39 ± 0.11 ^{bc}	2.14 ± 0.01 ^{bc}
PPE_A	73.50 ± 0.62 ^{a}	13.73 ± 0.62 ^{a}	4.70 ± 0.10 ^{a}	2.15 ± 0.03 ^{bc}
C_V	73.48 ± 0.62 ^{a}	15.03 ± 0.72 ^{a}	4.49 ± 0.16 ^{ab}	2.36 ± 0.11 ^{a}
BHT_V	73.82 ± 0.24 ^{a}	14.39 ± 0.41 ^{a}	4.25 ± 0.09 ^{bc}	2.08 ± 0.05 ^{c}
PPE_V	73.22 ± 0.45 ^{a}	14.82 ± 0.46 ^{a}	4.47 ± 0.08 ^{ab}	2.27 ± 0.05 ^{ab}

Mean±SD. Different letters in columns indicate significant differences (p<0.05). Aerobic (A) and vacuum (V) packaging.

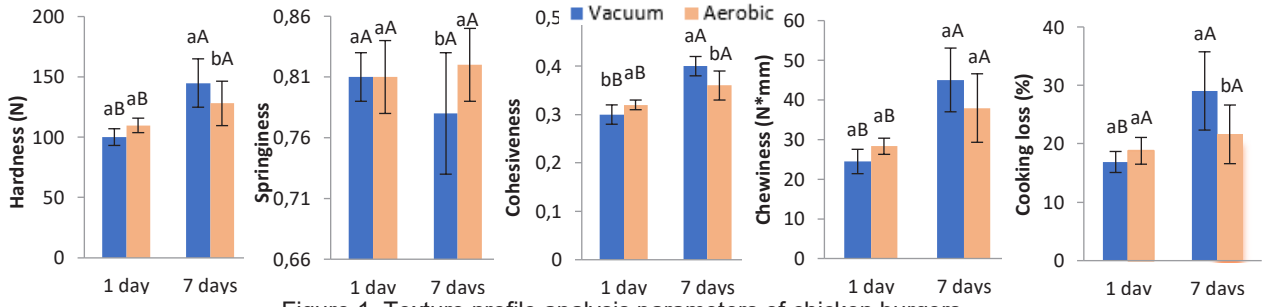

Figure 1. Texture profile analysis parameters of chicken burgers.
Different lowercase letters among treatments and capital letters among days indicate significant differences (p<0.05).

IV. CONCLUSION

The PPE did not promote relevant changes in the composition of the chicken burgers. Overall, the antioxidants also had no effect on TPA, cooking loss, and diameter reduction, which were influenced by the packaging and storage time of the samples. This study demonstrated that the use of pink pepper extract did not affect important quality parameters of the burgers, suggesting that PPE presents interesting possibilities as an antioxidant in the development of more natural meat products.

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Effects of hyaluronic acid on gelatinization properties and in vitro digestibility of wheat starch

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I. INTRODUCTION

Starch gelatinization leads to the destruction of starch crystallization and the layered structure, resulting in the formation of continuous gels, which greatly impacts the texture or consistency of the starch-based product. In addition to gelatinization properties, starch digestibility is also an essential quality criterion. Reducing the degree of starch gelatinization decreases its digestibility during the initial stages of digestion. Inhibiting the activities of α-amylase and amyloglucosidase can delay starch digestion, reducing the rate of glucose absorption and lessening the rise of serum glucose levels. Hyaluronic acid (HA) can form intramolecular hydrogen bonds, creating a three-dimensional structure that traps water due to its excellent water absorption properties. Consequently, HA's properties may influence the rheological and digestive characteristics of starch. Treating animal feed with hyaluronic acid to reduce the pasting and digestibility of starch is an innovative approach that can enhance animal welfare and performance. By controlling the rate of starch digestion, it is possible to manage energy levels, improve gastrointestinal health, and tailor diets more closely to the metabolic needs of different animals, potentially improving their overall health and productivity.

II. MATERIALS AND METHODS

The wheat starch (WS, 99% purity) with a carbohydrate content of up to 85.48% and a water content of 10.07% was provided by Xinliang Flour Co., Ltd (Jiangsu, China). The HA were purchased from Baiyao Biotechnology Co., Ltd (Jiangsu, China) with an average molecular weight of 349.7±3.5 kDa and a polydispersity index (PDI, Mw/Mn) of 1.025. The chemical reagents were analytical grade.

The content of leached amylose was determined according to the method of Kong et al. (2022) [1], with some modifications. The thermal properties of WS and WS-HA were determined using a differential scanning calorimeter. Rheological properties were tested using a Fourier infrared spectrometer. Starch digestibility was determined according to Zhou et al. (2022) [2].

The study's results were analyzed using SPSS 25.0 software (SPSS Inc., Chicago, USA) and presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), followed by the Duncan and Dunnett tests for multiple comparisons, was used to evaluate significant differences between groups. A p-value of < 0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

As shown in Table 1, the amount of leached amylose decreased with the increase of HA, indicating that HA could hinder the gelatinization process. With the increase of HA content, the consistency index (K) increased gradually, indicating that the addition of HA could reduce the viscosity of gelatinized starch and make it more fluid. The DO values of WS-HA increased with increasing HA concentration.

During digestion, the presence of HA had a noticeable impact on the starch fraction contents of WS (Figure 1A). The rapidly digestible starch (RDS) content decreased sharply from 60.20% to 47.78%, while there were notable increases in the levels of slowly digestible starch (SDS, from 13.05% to 21.52%) and resistant starch (RS, from 26.75% to 31.16%) in WS-HA gels as the concentration of HA increased ($p < 0.05$). This indicated a strong concentration-dependent suppressive effect of HA on WS digestion. The fluorescence intensity of α-amylase increased in the presence of HA (Fig. 9A and B), while the fluorescence intensity of amylosidase decreased slightly (Figure 1B and C). Based on the above results, the limited interactions of HA with enzymes indicate that its reduction of starch digestibility is mainly due to the formation of a physical barrier on the food surface and the inhibition of starch gelatinization.

Table 1 – Leached amylose, Gel properties, and DO values WS, WS-HA mixtures.

Samples	Leached amylose (%)	K (Pa·s^n)	n	R^2	R1047/1022
WS	12.61±0.26^a	14.989±0.221^d	0.254±0.005^{bc}	0.983	1.05
WS-HA-120:1	10.24±0.36^b	26.576±0.650^c	0.236±0.012^{cd}	0.996	1.07
WS-HA-24:1	8.95±0.15^c	35.659±0.012^b	0.267±0.001^{ab}	0.966	1.07
WS-HA-12:1	8.51±0.23^c	36.929±0.108^b	0.276±0.012^{ab}	0.975	1.10
WS-HA-6:1	8.15±0.03^c	40.895±0.646^a	0.282±0.007^a	0.986	1.18

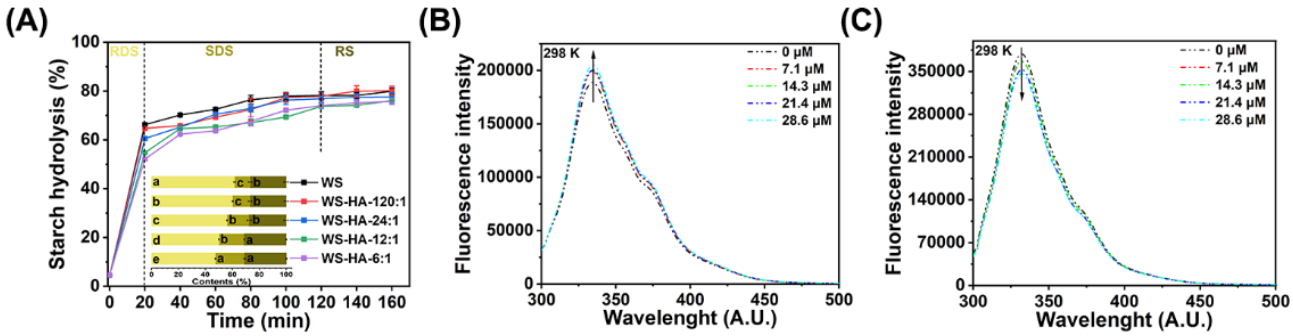

Figure 1. (A) Enzymatic hydrolysis curves and starch fraction distribution of the WS and WS-HA mixture. Letters indicate significant group differences ($p < 0.05$). Fluorescence quenching spectra of α-amylase (B) and amyglucosidase (C) in the presence of different concentrations of HA at 298 K.

CONCLUSION

In this study, the addition of HA inhibited starch gelatinization, increased the viscoelasticity and viscosity of WS-HA gels, and enhanced the ordering and stability of their network structure with increasing HA concentration. HA primarily reduces the degree of starch digestion by inhibiting starch gelatinization and preventing contact between enzymes and starch. These results can facilitate the use of HA in wheat starch foods.

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DETERMINING THE TEXTURE OF CHARQUI MADE FROM MATURED, DEFROSTED, AND SALTED BEEF USING COMMON SALT (NaCl)

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I. INTRODUCTION

The term 'charqui' originates from the Quechua word 'ch'arki', with ancestral roots. It refers to a product made from lean meat, typically cut into thin strips or chunks, salted dry or in brine, and sun-dried [1; 2]. The English term 'jerky' likely derives from charqui and denotes a sun-dried, salted, nitrite-cured meat product [1; 3; 4]. This category also encompasses various meat products from diverse origins, such as 'carne de sol' from northeastern Brazil, 'carne seca' from Mexico, 'cecina' from Spain, 'biltong' from South Africa, 'kilishi' from Nigeria and the African Sahara, 'kaddid' from Africa and South Asia, 'pastirma' from the eastern Mediterranean Sea, and 'bresaola' from Italy [1; 5; 6; 7].
Nguyen and Nguyen [8] highlight that the traditional sun-drying method primarily involves direct solar radiation, ensuring beef slices are cut uniformly for consistent drying.
The aim of this study was to produce and characterise charqui from matured, thawed, and salted meat using common salt, and to determine its texture.

II. MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Physical Properties of Biomaterials at the Faculty of Agricultural Engineering, University of Concepción, Chillán Campus.

For this study, three cuts from the hindquarter were utilized: the Striploin (LL), corresponding to the longissimus lumborum muscle (from the 10th rib to the lumbar vertebrae); the Eye of Round (PG), located at the back of the thigh, corresponding to the semitendinosus muscle; and the Sirloin Tip (PR), corresponding to the quadriceps femoris muscle.

Regarding texture, pieces from each charqui slice, measuring 3 cm^2 in area and 2 mm in thickness, were cut longitudinally to the muscle fibers. The value was measured using a Kramer® brand press in the INSTRON® Universal Test Equipment model 4467 H1998, with control over the area and thickness of each sample. The lowering speed was set at 10.00 $mm*min^{-1}$. Three repetitions of each cut were performed for each day of ripening. The same procedure was carried out for Equs® charqui, with three replicates.

For statistical analysis, a repeated samples experimental design was employed, considering time as a treatment factor for all commercial beef cuts. The assumptions of normality and homogeneity were previously analyzed using the Shapiro Wilk and Bartlett's tests respectively, with the data showing a normal distribution. The analysis was performed using descriptive statistics, analysis of variance, and the Kruskal-Wallis test, using the statistical program InfoStat Version 2019e.

III. RESULTS

Table 1 presents the values of maximum strength (N), slope ($N*mm^{-1}$), and hardness (N*mm) for LL charqui, PG, and PR, across different days of ripening. It can be observed that the three parameters evaluated for LL charqui fluctuate over time. However, no significant difference is noted for maximum strength ($p>0.05$), slope ($p>0.05$), and hardness ($p>0.05$). Furthermore, these values do not significantly differ ($p>0.05$) from those of Equs® charqui.

Table 1 – The values for maximum strength (N), slope (N*mm-1), and hardness (N*mm) of beef charqui, prepared with striploin (LL), eye of round (PG), and sirloin tip (PR) cuts across various days of maturation, and their comparison with Equs®* charqui.

Day	LL			PG			PR		
	Maximum strength	Slope	Hardness	Maximum strength	Slope	Hardness	Maximum strength	Slope	Hardness
2	3202.69^{a*}	808.04^{a}	7069.21^{a}	1728.86^{a}	524.09^{a}	3930.64^{a}	3338.26^{cd}	942.39^{bcde}	7353.80^{d}
5	4022.82^{a}	896.80^{a}	7596.82^{a}	2563.76^{a}	876.06^{a}	4420.78^{a}	2794.64^{bcd}	888.55^{abcde}	5948.60^{cd}
10	3527.52^{a}	944.27^{a}	8397.70^{a}	3900.68^{a}	792.94^{a}	10306.72^{a}	2040.27^{abc}	698.49^{ab}	3766.37^{abc}
15	2965.11^{a}	948.69^{a}	5833.40^{a}	2417.45^{a}	680.05^{a}	5580.68^{a}	1346.98^{ab}	429.40^{ab}	2694.71^{ab}
20	2008.73^{a}	876.22^{a}	3192.37^{a}	2540.94^{a}	512.87^{a}	6881.08^{a}	3757.05^{cd}	1044.60^{cde}	5665.83^{bcd}
25	2825.51^{a}	780.80^{a}	6672.13^{a}	2358.39^{a}	506.95^{a}	5049.37^{a}	3217.45^{d}	1282.20^{e}	5141.99^{bcd}
30	2645.64^{a}	663.69^{a}	5998.06^{a}	2998.66^{a}	778.97^{a}	5807.34^{a}	1555.71^{abcd}	365.45^{ab}	3876.49^{abcd}
45	3935.58^{a}	1282.60^{a}	6887.22^{a}	1373.83^{a}	476.70^{a}	2896.73^{a}	2420.14^{abcd}	743.44^{abcd}	4535.70^{abcd}
60	2744.97^{a}	872.26^{a}	4819.57^{a}	1708.73^{a}	420.53^{a}	4875.01^{a}	3210.74^{d}	1168.10^{cde}	6433.29^{cd}
75	2989.27^{a}	807.91^{a}	7060.43^{a}	2481.88^{a}	489.48^{a}	6429.87^{a}	2159.07^{abcd}	588.32^{abc}	4393.23^{abcd}
90	2934.23^{a}	1106.30^{a}	4449.14^{a}	1372.49^{a}	385.78^{a}	3032.10^{a}	910.07^{a}	308.38^{a}	1801.95^{a}
Equs®	3600.00^{a}	1367.60^{a}	6300.15^{a}	3600.00^{a}	1367.60^{a}	6300.15^{a}	3600.00^{cd}	1367.60^{de}	6300.15^{bcd}

Different letters apply to the same column and medians with a common letter are not statistically different (p>0.05) in the Kruskal Wallis test.
*Equs®: Commercial jerky made from unripened beef.

IV. CONCLUSION

The importance of texture in the preparation of charqui should be emphasised, as it allows for the optimisation of resources. In the three cuts of meat, the values of the parameters analysed fluctuated over the course of the maturation period and there was no significant difference for maximum strength (p>0.05), slope (p>0.05) and hardness (p>0.05).

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COMPARATIVE STUDY ON THE EFFECTS OF RABBIT AND CHICKEN MEAT ON THE PHYSICOCHEMICAL PROPERTIES OF LOW-FAT BURGERS WITH AMARANTH FLOUR

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I. INTRODUCTION

In recent years, research has increased to obtain healthier, safer, more sustainable meat products with wide acceptance to satisfy growing consumer demand. Rabbit meat is a food rich in nutrients, with a lower environmental impact than red meat and chicken. It has a lower fat, and cholesterol than other meats, is highly digestible, and has a more unsaturated fatty acid profile [1]. However, it is not widely consumed by Brazilians. Amaranth is a pseudo cereal rich in vitamins and minerals and contains high-biological-value proteins. Due to its neutral sensory profile, amaranth flour can be included in meat products, improving its nutritional value by incorporating essential elements for bone, muscle, and metabolic health [2]. Thus, this study aimed to compare the effects of rabbit and chicken meat on the physicochemical and technological properties of burgers with partial fat reduction and the addition of amaranth flour in search of a healthier alternative to traditional options.

II. MATERIALS AND METHODS

The rabbit meat used to produce the burgers was kindly donated by the Federal University of Viçosa (Brazil), the chicken meat was purchased from a local market in Sete Lagoas (Brazil), and the other ingredients were obtained from New Max Industrial (Brazil). Two control treatments with 70.0% rabbit (FRC) and chicken meat (FCC), 15% pork back fat, and without amaranth flour (AF) were elaborated, and two treatments with 30% fat reduction and 4.5% AF, with rabbit and chicken meat, denominated respectively FRA and FCA. All treatments had 1.0% soy protein isolate, 1.15% spices, 1.7% sodium chloride, 0.05% sodium erythorbate, 0.3% sodium tripolyphosphate, and 10.8% cold water. The burgers were prepared according to Essa & Elsebaie [3]. The analyses included moisture, protein, and ash [4], lipid content [5], and instrumental color determination. Carbohydrate content was calculated by difference. The burgers' technological properties (cooking losses, shrinkage, moisture retention) and texture profile were also evaluated (hardness, springiness, cohesiveness, chewiness). The results were assessed using analysis of variance (ANOVA) with general linear models, considering the treatments as a fixed effect and the experiment replications as a random term (n = 3), using Statsoft. Inc. version 7 software (TIBCO Software Inc., California, USA). Tukey's test at 5% significance level ($P \leq .05$) was used to determine significant differences between treatments.

III. RESULTS AND DISCUSSION

Table 1 shows the burgers' approximate composition, and technological characteristics. Adding AF reduced the protein and fat content and increased the carbohydrate content. Besides, rabbit burgers had a higher protein and lower lipid content than chicken burgers, possibly attributed to the to the composition of rabbit meat. A fat content reduction of at least 15.3% was observed in FRA, which differed from the other treatments ($P < .05$). About the technological properties of the products, lower cooking losses and greater moisture retention were observed in samples with AF. Amaranth (rich in proteins and starch) can be a binder because it increases water and fat retention. The samples with AF also showed lower shrinkage than controls ($P < .05$). Texture data (Figure 1) showed that the control samples (FRC and FCC) had greater cohesiveness and chewiness than the treatments with AF. Lower values of springiness ($P<.05$) were found in samples with rabbit meat and AF, and lower values of hardness were found in samples with chicken meat and AF, demonstrating that the amaranth had an impact on textural parameters. The effects of reformulation in color parameters and appearance of the products can be observed in Figure 2. The samples with AF (FRA and FCA) were redder than the controls; however, about the b* parameter, the type of meat also influenced the results. In chicken

treatments, amaranth left the samples more yellowish; in rabbit meat samples, AF reduced the yellow tone. Regarding luminosity, amaranth flour provided a darker appearance for the rabbit meat treatment (FRA) and did not influence the chicken meat treatments (P>.05).

Table 1 – Chemical composition and technological properties of chicken and rabbit grilled burgers formulated with amaranth

Parameter	FRC	FCC	FRA	FCA
Chemical composition grilled samples				
Moisture (%)	50.37±0.19^{a}	50.94±0.36^{a}	50.65±0.83^{a}	48.81±0.47^{b}
Protein (%)	27.72±0.35^{a}	26.19±0.24^{b}	25.69±0.55^{b}	24.19±0.21^{c}
Fat (%)	14.68±0.10^{b}	15.55±0.20^{a}	12.44±0.19^{c}	14.22±0.24^{b}
Ash (%)	5.16±0.03^{a,b}	5.26±0.04^{a}	4.73±0.02^{b,c}	4.61±0.51^{c}
Carbohydrates (%)	2.07^{b}	2.06^{b}	6.5^{a}	8.17^{a}
Tecnological parameters				
Cooking losses (%)	40.18±4.45^{a}	39.58±2.40^{a}	33.08±3.23^{b}	33.96±1.77^{b}
Shrinkage (%)	22.95±1.76^{a}	8.64±3.02^{c}	15.63±4.84^{b}	6.65±2.58^{c}
Moisture retention (%)	29.53±2.69^{b}	30.78±1.22^{a,b}	33.90±1.64^{a}	33.99±2.28^{a}

^{a,b,c} Mean values within the same line horizontally followed by the same lowercase letters did not show any significant difference (P > .05) by Tukey's test. FRC: control with rabbit meat; FCC: control with chicken meat; FRA: 30% fat reduction and 4.5% AF with rabbit meat; FCA: 30% fat reduction and 4.5% AF with chicken meat.

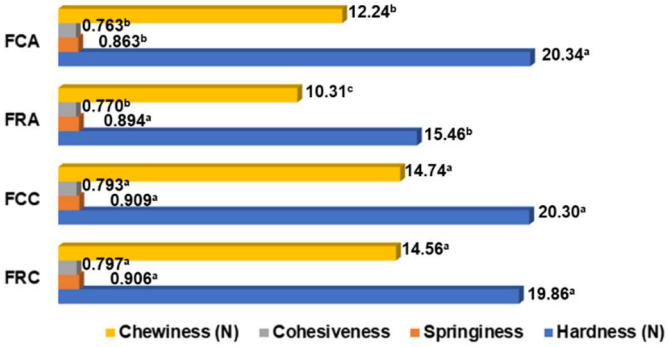

Figure 1. Texture parameters of chicken and rabbit grilled burgers with amaranth.
^{a,b,c} Mean values next to bars of the same color followed by the same lowercase letters did not show any significant difference (P>.05) by Tukey's

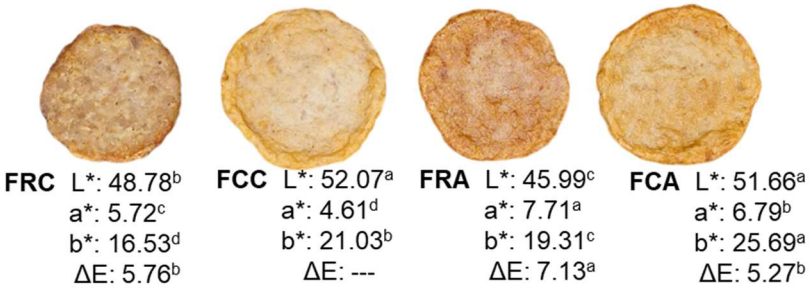

Figure 2. Appearance and color parameters of chicken and rabbit grilled burgers with amaranth.
^{a,b,c,d} Mean values within the same line horizontally followed by the same lowercase letters did not show any significant difference (P > .05) by Tukey's test. L: brightness; a*: green-red; b*: blue-yellow; ΔE: euclidean distance

IV. CONCLUSION

Rabbit burgers can be promising meat product options due to their higher nutritional quality, more sustainable production, and technological properties, similar to traditional options such as products made with chicken meat. Amaranth flour improved the technological properties of burgers and enabled a partial reduction in fat. Further studies are desirable to assess consumers' acceptability of this type of product and promote rabbit meat consumption among the Brazilian population.

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STRUCTURE, TEXTURE, AND SENSORY PROPERTIES OF HYBRID MORTADELLA WITH WHOLE AND DEFATTED CRICKET (*GRYLLUS ASSIMILIS*) FLOUR

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I. INTRODUCTION

Edible insects have stood out worldwide as potential solutions to improve food security and dietary diversity, demonstrating that they can be viable substitutes for conventional protein sources due to their high nutritional value, biofunctional properties, and environmentally sustainable rearing methods [1]. The development of hybrid meat products with insect proteins is a promising trend to reduce the consumption of traditional animal protein and increasing the acceptance of alternative protein sources, especially in Western society. Therefore, the study investigated the impact of replacing lean meat with cricket (*Gryllus assimilis*) flour, whole and defatted, on the structural and sensory characteristics of mortadella.

II. MATERIALS AND METHODS

Beef (*Quadriceps femoris*, 72.3% moisture, 20.8% protein, 4.2% fat and 1% ash), pork (*Longissimus thoracis et lumborum*, 69.4% moisture, 7.8% lipids, 21.2% protein, and 1.5% ash), and pork back fat (10.8% moisture, 82.3% lipids, 6.05% protein, and 0.44% ash) were used to produce mortadella. Hakkuna (Brazil) kindly provided the dehydrated crickets (*Gryllus assimilis*), that were ground in a hammer mill and then sieved through 20 mesh meshes. Lipid extraction of flour was carried out according to the method of Ndiritu et al. (2017) [2] with some modifications. Five treatments were prepared with 5 and 10% replacement of lean meat with WCF (whole cricket flour) and DCF (defatted cricket flour), respectively, F5W, F10W, F5D, F10D, besides the control (FC), with 60% meat. All treatments had 0.015% sodium nitrite, 1.58% spices, 1.5% sodium chloride, 0.05% sodium erythorbate, 0.5% sodium tripolyphosphate, and 16.35% ice. The products were prepared according to Câmara & Pollonio [3]. Texture profile analysis (TPA) was evaluated (25 ºC) in a TA-xT2i texture analyzer, with eighteen cubes (20 mm) that were axially compressed (2 cycles of 30% compression, probe P35) at a constant speed of 1 mm/s. The parameters evaluated were hardness (N), springiness, cohesiveness, and chewiness (N). To evaluate the microstructure, the samples (1 cm^2 and thickness of 0.2 cm) were freeze-dried and analyzed in a scanning electron microscope TM 4000 Tabletop Microscope (Hitachi Technologies, Japan), using an acceleration of 15 kV in Analy mode. The images were obtained with an increase of 200x. After approval of the study (CAAE 67415023.2.0000.5151) by the Research Ethics Committee of the Federal University of São João del-Rei, the samples were subjected to sensory acceptance and CATA tests with 89 consumers. The results were assessed using analysis of variance with general linear models, considering the treatments as a fixed effect and the experiment replications as a random term (n = 3), using Statsoft. Inc. version 7 software (TIBCO Software Inc., California, USA). Tukey's test ($P < 0.05$) was used to determine significant differences between treatments.

III. RESULTS AND DISCUSSION

Cricket flour influenced considerably the textural parameters (Table 1). Adding WCF decreased the firmness, cohesiveness, and chewiness values, and F10W differed from all other treatments ($P < 0.05$), while adding DCF increased the chewiness values. These results are in consonance with the microstructural images (Figure 1). Figure 1 has shown that F10W presented a very heterogeneous, spongy, and more discontinuous structure with coalescence of the fat globules. In FC, a more cohesive structure is observed with larger fat globules with well-defined and delimited borders, which are characteristic of emulsified meat products. Figure 2 (A, B, C) presents the results of the acceptance test and CATA. The F10W treatment was removed from sensory studies because it was characterized by high instability of the meat emulsion in previous studies. Data from the acceptance test (Figure 2A) showed that the treatment with the highest level of replacement of meat with cricket flour (F10D) exhibited lower values for all attributes and differed ($P < 0.05$) from the other treatments. Consumers

had a similar perception of the mortadella samples with WCF and DCF at levels of 5%, as there were no differences from the control, except for color. CATA results (Figure 2B) show that FC and F5W were perceived to have a mortadella flavor, aroma, and ideal color. The study also indicated that F5D was described as having an ideal amount of salt, texture, and juiciness, which aligns with instrumental texture data. In contrast, F10D was associated with the terms low juicy and sandy. Figure 2C demonstrated that the attributes of juicy, ideal color, ideal texture, and ideal amount of salt were associated with the overall liking of the mortadella.

Table 1 – Texture parameters of mortadella with partial replacement of lean meat by cricket flour

	FC	F5D	F10D	F5W	F10W
Hardness (N)	14.81±0.95^{a}	14.95±0.77^{a}	15.79±0.54^{a}	13.29±0.68^{b}	11.71±0.50^{c}
Springiness	0.94±0.01^{a}	0.93±0.01^{c}	0.93±0.00^{b,c}	0.94±0.01^{a,b}	0.92±0.01^{c}
Cohesiveness	0.84±0.01^{a}	0.80±0.00^{b}	0.81±0.00^{b}	0.81±0.01^{b}	0.79±0.01^{c}
Chewiness (N)	10.91±0.85^{b,c}	11.63±0.62^{b}	12.65±0.70^{a}	10.38±0.26^{c}	8.58±0.30^{d}

* The values represent the mean ± standard deviation. ^{a,b,c,d} Means in the same row with different letters indicate significant differences ($P < 0.05$). FC: control with 60% of meat; F5D: 5% replacement of lean meat with DCF; F10D:10% replacement of lean meat with DCF; F5W: 5% replacement of lean meat with WCF; F10W: 10% replacement of lean meat with WCF.

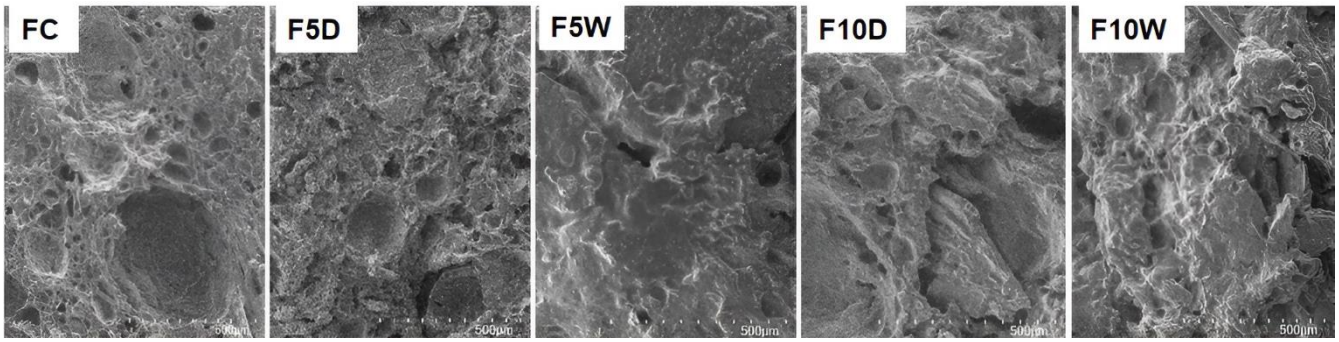

Figure 1. SEM images of mortadella with WCF, DCF and control (200 x magnification, Scale bar =500 µm)

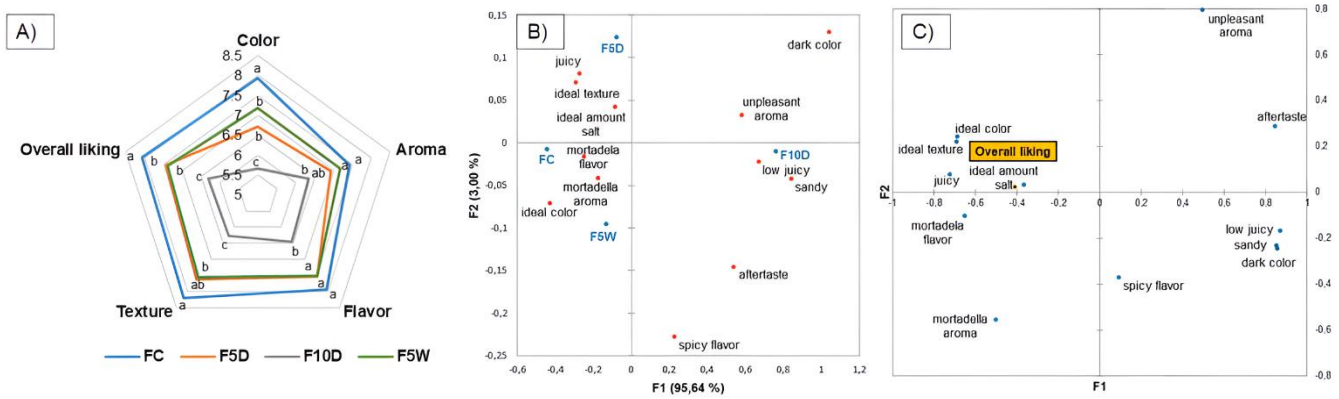

Figure 2. (A) Sensory acceptance of mortadella with partial replacement of lean meat by cricket flour. Equal letters in the same attribute are not statistically different ($p>0.05$). (B) Representation of samples and attributes in the first two dimensions of correspondence analysis of mortadella samples using CATA questions. (C) Correlation between sensory attributes and overall liking in the first two dimensions of principal coordinate analysis.

IV. CONCLUSION

Replacing up to 5% of lean meat with whole and defatted cricket flour may be a relevant strategy to produce hybrid meat emulsion with adequate sensorial, microstructural, and textural properties.

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PSYLLIUM (*PLANTAGO OVATA FORSK*) GEL AND EMULSION GEL AS FAT REPLACERS IN PHOSPHATE-FREE AND LOW SALT SAUSAGES

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I. INTRODUCTION

Sodium tripolyphosphate is an important additive for the meat industry due to its ability to increase protein solubility and water retention [1]. However, due to the health issues associated with consumption of phosphates, their reduction or total replacement has been studied. Fibers such as psyllium (*Plantago ovata Forsk*) are considered promising alternative to replace additives, sodium chloride, the main source of sodium, and fat in meat products. This study investigated the effect of psyllium gel and emulsion gel in the physicochemical and microstructural properties of phosphate-free meat emulsions with reduced fat and sodium content.

II. MATERIALS AND METHODS

Two distinct strategies were developed to replace pork backfat. The first strategy was based on using emulsion gels containing water, vegetable oil, and psyllium (EGs) to substitute 100% of the backfat. The second one used only psyllium gels (PG) containing water and this prebiotic fiber aiming to reduce the backfat by 50%. Emulsion gels (EGs) were produced with psyllium (6% and 12%), canola oil (40%) and water in a homogenizer (Thermomix TM5). Gels (PG) were produced with psyllium (15% and 20%) and water using a mixer (Electrolux, 400W power, turbo function). Both were stored at 4 °C for 24 hours until use. Six phosphate-free treatments were developed with a 25% reduction in sodium chloride (NaCl) (1.5 g/100g), 62% beef, 0.015% sodium nitrite, 0.57% spices, 0.05% sodium erythorbate, and ice (FC1: 15.87%; FC2: 25.87%; F1 and F2: 15.87%; F3 and F4: 5.86%). Two control treatments, FC1 and FC2, with 20% and 10% of pork backfat, respectively. F1 and F2 with 100% of fat replacement by EG (produced with 6% and 12% of psyllium, respectively), and F3 and F4 with 50% of fat replacement by PG (produced with 15% and 20% of psyllium, respectively). Sausages were produced according to Felisberto et al. 2015 [2]. Emulsion stability (ES) [3], cooking loss [4], pH (MA 130 Metler pH meter), water activity (a_w) (Aqualab, Decagon Devices, INC., PULLMAN, USA), and scanning electron micrographs (SEM) were analyzed. The results were assessed using analysis of variance (ANOVA) using IBM SPSS Statistics 20 software. Tukey's test ($P < 0.05$) was used to determine significant differences between treatments.

III. RESULTS AND DISCUSSION

Table 1 shows that the absence of phosphates, the fat and NaCl reduction, mainly affected FC2. This was expected, since the lack of a structuring agent reduces the water retention capacity (WRC) of the meat system [5]. The samples containing EGs (F1 and F2) and PG (F3 and F4) showed lower liquid loss, which was not influenced by the absence of phosphate or by fat and sodium reduction, which may be associated with the hydrophilic and gelling properties of psyllium [6]. FC1 had the lowest cooking loss ($P < 0.05$) than other sausages, probably due to role of animal fat in the technological properties of meat emulsion. Sausages with PG (F3 and F4) also presented a good liquid retention, which may be related to the quantity and functional properties of psyllium fibers.The pH of the sausages varied from 6.28 to 6.19, and was affected by the lipid reformulation. The presence of EGs and PG had an impact on reducing the pH, which was expected due to pH values of psyllium (6.06). The a_w values ranged from 0.9884 to 0.9789. Despite the differences observed, all the treatments showed values typical of an emulsified meat product.

Table 1 – Effect of PG and EG on phosphate-free sausages with reduced sodium and fat content

	ES (%)	Cooking loss (%)	pH	a_w
FC1	2.73 ± 0.38^{b}	11.12 ± 1.10^{d}	6.28 ± 0.01^{a}	0.9820 ± 0.001^{b}
FC2	11.60 ± 0.77^{a}	18.11 ± 1.59^{a}	6.26 ± 0.01^{b}	0.9818 ± 0.001^{b}
F1	1.87 ± 0.54^{c}	17.77 ± 1.58^{a}	6.22 ± 0.01^{c}	0.9814 ± 0.001^{b}
F2	0.03 ± 0.01^{d}	16.39 ± 1.94^{b}	6.19 ± 0.01^{d}	0.9833 ± 0.001^{a}
F3	0.02 ± 0.01^{d}	14.22 ± 1.26^{c}	6.26 ± 0.01^{c}	0.9784 ± 0.001^{c}
F4	0.00 ± 0.00^{d}	14.15 ± 1.43^{c}	6.26 ± 0.01^{c}	0.9789 ± 0.001^{c}

Means in the same column with different letters indicate significant differences ($P < 0.05$). FC1: 100% pork backfat; FC2: 50% pork backfat; F1: replacement of 100% pork backfat with EGs produced with 6% psyllium (1.2% Psyllium in the final product); F2: replacement of 100% of pork backfat with EGs produced with 12% psyllium (2.4% Psyllium in the final product); F3 - replacement of 50% pork backfat by PG (gel at 15% concentration - 3% Psyllium in the final product); F4: replacement of 50% pork backfat by PG (gel at 20% concentration - 4% Psyllium in the final product).

As shown in the micrographs (Figure 1), both strategies, samples with EGs (F1 and F2) and those with PG (F3 and F4) exhibited continuous and compact structures.

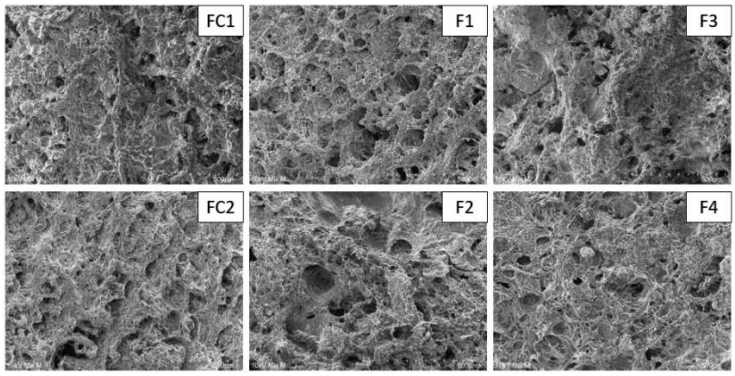

Figure 1. Scanning electron micrograph (SEM) images of sausages (magnification 100×, scale bar = 500 µm).

IV. CONCLUSION

Structured gels of psyllium are a promising non-meat ingredient for use in emulsified meat products, standing out as an effective substitute for phosphate salts in low-fat and sodium products. Its incorporation can make meat products healthier, in line with demands for clean label options.

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Effect of the addition of collagen to Italian type salami on color and moisture parameters

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I. INTRODUCTION

Collagen is a structural protein that can be found in the dermis, bones, tendons, cartilage, ligaments, and other tissues, including the organs of animals [1]. It has several industrial applications, due to its characteristics, such as neutral odor, colorless, low viscosity in aqueous solution, acts as a stabilizer and emulsifier, as well as forming foam and films [2]. In meat products, the addition of collagen improves the gelling capacity, water retention, and protein content, as well as the sensory properties of the products [3]. The aim of this study was to evaluate the effects of the collagen fiber addition to Italian type salami with salt reduction on moisture and instrumental color.

II. MATERIALS AND METHODS

The salami was produced by mixing the ingredients in an automatic blender (Frigomaq, Chapecó, Brazil) for 10 minutes. Then, the samples were stuffed in collagen cases (diameter of 70 mm) of 20 cm and conducted to maturation for about 29 to 47 days at a temperature of 15-25 °C and relative humidity of 75-95 %, until a water activity of 0.90 has been reached. Therefore, the ripening time was different between the treatments. Six treatments were carried out, three with 2.5% sodium chloride (NaCl): without collagen - **CON**, 0.25% collagen - **T25**, 0.5% collagen - **T50**. And three with 1.25% NaCl: without collagen - **TR**, 0.25% collagen - **TR25**, 0.5% collagen **TR50**. The moisture content was determined according to the official method of the AOAC [4]. A Colorflex 45/0 spectrophotometer (HunterLab, Reston, USA) was used to determine the color parameters through the CIELAB system of color specifications. The results were analyzed for analysis of variance (ANOVA two ways) using the General Linear Model (GLM) and the means were compared using post-hoc Tukey test ($p < 0.05$) with STATISTICA 7.0 (StatSoft, Inc., 2004) software.

III. RESULTS AND DISCUSSION

The addition of collagen (0.50 %) contributed to an increase ($p < 0.05$) in the moisture in Italian type salami on day zero (Table 1), where TR50 presented the higher moisture value and it was similar to T50 and CON (67.07 %, 65.79 % and 62.54 %, respectively).

Table 1. Moisture of Italian type salami (%).

Treatments	Days							
	0	3	13	23	33	40	43	47
CON	62.54^{ab}	55.57	47.78^{a}	42.53	37.75^{b}	-	-	-
T25	61.15^{b}	56.20	47.93^{a}	44.84	38.97^{ab}	-	-	-
T50	65.79^{ab}	56.33	44.66^{b}	42.36	-	-	-	-
TR	60.98^{b}	55.85	49.01^{a}	43.44	39.04^{ab}	36.23	35.58^{b}	34.87
TR25	61.80^{b}	57.10	48.79^{a}	45.54	40.00^{a}	36.83	35.55^{b}	-
TR50	67.07^{a}	56.14	48.13^{a}	42.98	40.47^{a}	39.23	38.63^{a}	-
SEM	0.604	0.539	0.412	0.371	0.287	0.619	0.484	-
p-value	0.002	0.984	0.001	0.057	0.014	0.117	0.000	-

$^{a-b}$ Means followed by different letters in the same column differ by Tukey Test ($p \leq 0.05$); SEM – standard error of the mean. CON – without collagen and 2.5 % NaCl; T25 – 0.25 % collagen and 2.5 % NaCl; T50 – 0.5 % collagen and 2.5

% NaCl; TR – without collagen and 1.25 % NaCl; TR25 – 0.25 % collagen and 1.25 % NaCl; TR50 – 0.5 % collagen and 1.25 % NaCl.

The same concentration provided higher ($p < 0.05$) moisture for T50 at 13 days and for TR50 at 43 days when this treatment was ready for consumption with ideal water activity and higher moisture than TR ($p < 0.05$), which reached optimum water activity only at 47 days.

The L* values ranged from 54.40 to 56.09 and no difference was observed ($p > 0.05$) between the treatments of the Italian type salami (Table 2). The 50% reduction in NaCl and the addition of 0.5% collagen (TR50) provided the highest a* values with greater redness than the CON, T25 and T50 treatments ($p < 0.05$). In addition, TR50 showed the lowest whiteness value, different from the CON and TR treatments. The reduction of NaCl increased the b* value of Italian type salami, with the TR, TR25 and TR50 treatments being similar to each other but different from CON, T25 and T50 ($p < 0.05$) and collagen did not affect on this parameter.

Table 2. Color parameters of Italian type salami.

Treatments	L*	a*	b*	Whiteness
CON	55.87	8.95^{c}	9.15^{b}	54.03^{a}
T25	55.02	9.52^{bc}	9.56^{b}	53.01^{ab}
T50	54.55	9.55^{bc}	9.44^{b}	52.57^{ab}
TR	56.09	9.86^{ab}	11.11^{a}	54.09^{a}
TR25	55.54	9.80^{ab}	10.59^{a}	53.24^{ab}
TR50	54.40	10.32^{a}	10.80^{a}	52.01^{b}
SEM	0.194	0.068	0.078	0.199
p-value	0.056	0.000	0.000	0.014

$^{a-c}$ Means followed by different letters in the same column differ by Tukey Test ($p \leq 0.05$); *SEM* – standard error of the mean. CON – without collagen and 2.5 % NaCl; T25 – 0.25 % collagen and 2.5 % NaCl; T50 – 0.5 % collagen and 2.5 % NaCl; TR – without collagen and 1.25 % NaCl; TR25 – 0.25 % collagen and 1.25 % NaCl; TR50 – 0.5 % collagen and 1.25 % NaCl.

IV. CONCLUSION

The results show that the reduction of NaCl did not affect the moisture content but increased the ripening time of Italian salami. The addition of collagen, especially at a concentration of 0.5%, has the potential to contribute to the moisture and color parameters of Italian type salami, such as a* value and whiteness.

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The Development and Evaluation of Naturally Cured Lamb Ham

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I. INTRODUCTION

"Natural" or organic foods and ingredients are currently a growing trend in the current U.S food market. Natural curing agents derived from nitrate rich vegetables (celery, spinach, beets) can be used to replace curing salts that contain sodium chloride and sodium nitrite [1]. Vegetables can be fermented into solutions and/ or dried into powder to convert sodium nitrate to sodium nitrite (Jo et al., 2020). The fermented vegetables were tested on pork and showed properties like curing salt on color changes, quality, shelf life, and food safety [2, 3, 4]. Currently, celery powder is commonly used in "uncured" meat products in the U.S market. Swiss chard is a nitrate-rich vegetable with potential for use as meat a curing agent. The current study evaluated both Swiss chard and celery as a pre-converted powder (powders with sodium nitrate converted to sodium nitrite) for use as a dried rubbed on lamb. The objective of this was to compare the effects of vegetable powders (celery and Swiss chard) on the quality characteristics i.e., color, water activity (aw), pH, residual nitrite levels, and textural properties of lamb hams.

II. MATERIALS AND METHODS

Lamb legs were dry cured using a two-part process (curing and salt equalization) with rubs containing varying levels of Swiss chard and celery compared to curing salt. Three different rubs used in this study which contained curing salt (Prague salt) or one of the two natural vegetable powders (containing sodium nitrite) namely, Swiss chard powder (SC) and celery powder (CP). The curing agents were added to obtain 327 ppm nitrites in control rub, 250 and 500 ppm nitrites in SC and 250 and 500 ppm nitrites in CP. These rubs were applied by rubbing on it on the surface as dry-ingredient mixes. The dry rub mixes were applied to obtain a final concentration of sodium nitrite below 100 ppm in the finished product. Any excess rub mix was removed either brushed off by hand or washed off. The rubbed lamb legs were then placed in a cooler. at 2.8 ± 1°C and 70 - 80% (RH) for 9 days per kg. The lamb was rested on metal wire racks wrapped with 4 mil heavy duty plastic sheeting which had four rectangular cuts (15.24 cm x 2.54 cm) per each of the three rows. The lamb hams were briefly removed from the cooler on day 7 and 14 to apply the fresh rub, brushing and washing off previous rub with water before applying the next portion. They were returned to the cooler and now held at 12.8℃ ± 1°C. The lamb hams equalized for 4 days per kg. The lamb hams were finished after this stage. The finished lamb hams texture was evaluated with a texture profile analysis (TPA) test and a unique puncturable test.

III. RESULTS AND DISCUSSION

The water activity showed a significant difference when the CP (500ppm) treatment was compared to CP (250ppm) and the control. Over the processing time, the water activity decreased from 0.98 on day 0 to 0.89 on day 42 (Fig. 1C, 1D, and 1D). The progressive decrease in the water activity occurred in all the treatment groups as expected with dry curing with a slightly lower water activity in the celery powder groups. Figure 3 showed the CP500 treatment group decreasing slightly lower (about 0.89) than CP250, SC250, SC500, and control (0.91 to 0.92). The Swiss chard unlike the celery powder acted like the control regardless of the cure mixture. The different treatment groups show that celery powder and Swiss chard can be used to replace Prague salts (control) with a consideration of the other ingredients in the curing agents. Swiss chard lamb treated at 500 ppm of sodium nitrite created

the best final product compared to the control because the treatment had the most significantly similar values throughout all the analyses. The Swiss chard treated groups was the least not significantly different than the control group, but the celery powder treatment at 500 ppm showed various significant differences. The additional ingredients (salt and silica) in the celery powder impacted the qualities of the lamb hams when the celery powder was added at higher quantities. An adjustment in the formulation to reduce the amount of pure salt added would adjust for the quality effects caused by additional salt. The firmness (hardness) of the lamb ham was the significantly highest in CP500 and lower for SC250 and SC500 and CP250 and lowest for the control (Fig. 2).

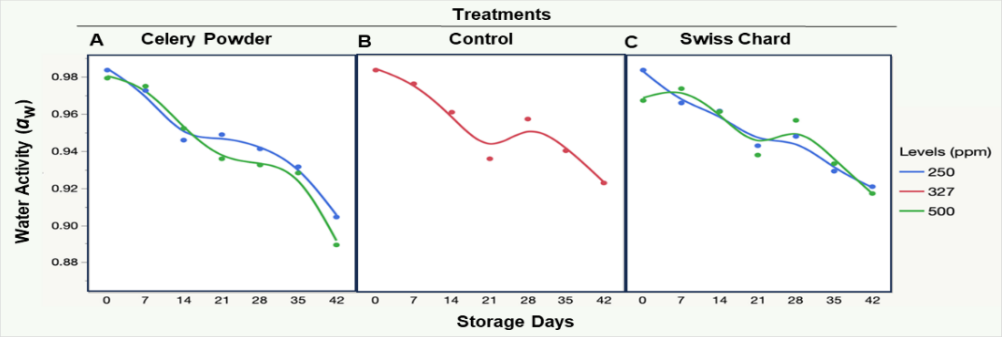

Figure 1. The images show the vegetable powders celery powder (A) and Swiss chard powder (B). The water activity decreased over time of lamb ham naturally cured with celery powder (C), control (D), and Swiss chard (E).

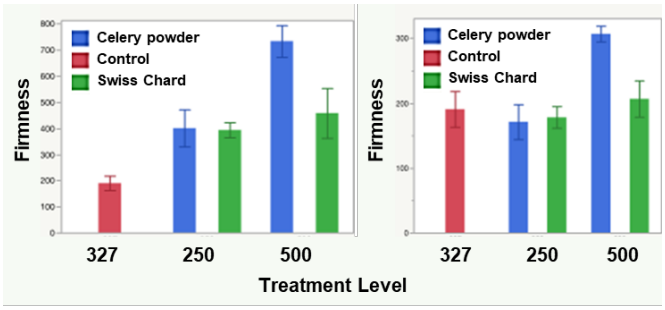

Figure 2. The hardness of final lamb ham shows the firmness (hardness) from the textural profile analysis and force required to fracture the final sliced lamb ham. The force was measured in grams (g).

CONCLUSION

Overall, the dry-curing process caused the expected decrease in water activity and redness development in the cured lamb hams. Further investigation of this group could result in a new high value dry-cured product for the U.S market as there is no dry-cured sliced lamb ham in the U.S market. Overall, a naturally cured lamb ham is a viable product that could enter the U.S market. Further tests for the success of a naturally cured lamb ham would first need to test the microbial safety followed by consumer acceptability tests. Future products could add aging or smoking tests to further process the meat and develop specific flavors.

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Innovative Processing of Chicken Heart Protein Hydrolysates: A Physico-Chemical Study Post Lactobacilli Fermentation

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I. INTRODUCTION

In the recent years, there is tremendous increase in production and consumption of chicken meat, and at the same time, production of slaughterhouse by-products has also been increased greatly. Unfortunately, by-products generated during processing are not properly utilized. Recent studies have indicated that chicken heart is a good source for protein hydrolysates having several biological properties, such as antioxidant, antimicrobial and others functional activities. Using proteolytic microorganisms to ferment food proteins is an emerging method for production and processing of protein hydrolysates on an industrial scale. In contrast to traditional enzymatic breakdown, fermentation emerges as a financially viable approach to produce food-grade protein hydrolysates and bioactive peptides. LAB fermentation represents an environmentally friendly and sustainable technology, wherein lactic acid bacteria (LAB) aid in waste preservation while recovering crucial biomolecules. This study aims to evaluate the freeze-dried chicken heart hydrolysates produced by LAB fermentation, focusing on their physico-chemical characterization.

II. MATERIALS AND METHODS

Freeze dried *Lactobacillus helveticus*-292 and *Latiplantibacillus plantarum*-025 were obtained from National Collection of Dairy Culture (NCDC), ICAR- National Dairy Research Institute (ICAR-NDRI), Karnal, Haryana. Chicken heart was hygienically obtained from the Post-Harvest Technology (PHT) section of ICAR-Central Avian Research Institute (CARI), Izatnagar, Uttar Pradesh. The bacterial count in working cultures was adjusted to 10^7 cfu/mL.Fermentation temperature was maintained at 37°C for 16 h. Freeze drying of hydrolysates was done at a temperature below -50°C and a pressure below 150mTorr in a Freeze dryer (iIShinBioBase, South Korea). The water activity was estimated using a digital water activity meter (4TE Dew Point water activity meter, Aqua lab, Malaysia). The Fourier Transform Infrared Spectroscopy was done using Ailent Cary 630 Spectrometer (California, United States) [1]. The particle surface morphology of the freeze-dried heart hydrolysates was studiedusing Scanning Electron Microscope (EMCRAFTS, South Korea) at an acceleration voltage of 20kV [2].

III. RESULTS AND DISCUSSION

Heart hydrolysates prepared with *L. helveticus* fermentation showed significantly ($p<0.05$) higher protein % than hydrolysates fermented with *L. plantarum*as shown in table 1.

Table 1:Physico-chemical properties of freeze-dried raw chicken heart (Hc) and chicken heart hydrolysates (2Hh16 and 2Hp16)

Parameters	Hc	2Hh16	2Hp16
Yield %	15.45±0.33^{c}	21.11±0.43^{a}	19.93±0.25^{b}
aw	0.56±0.009^{a}	0.47±0.005^{c}	0.51±0.001^{b}
Protein	73.75±0.80^{c}	84.41±0.34^{a}	81.33±0.51^{b}

n=6, Mean ± Standard Error bearing different superscripts within row differ significantly (P<0.05). Hc- Freeze-dried raw heart; 2Hh16- 2% *L. helveticus*, 16 h; 2Hp16- 2% *L. plantarum*, 16 h.

FTIR spectra for peak positions in freeze-dried raw heart (Hc) as well as for the heart hydrolysates (2Hh16 and 2Hp16) are shown in Fig 1a. Hc, 2Hh16 and 2Hp16 revealed their amide I peaks at 1639.00, 1638.06 and 1639.78cm^{-1}, respectively. Higher wavenumber coupled with higher amplitude of amide I band of 2Hp16 than Hc indicated towards the comparatively higher

disintegration of molecular structure in 2Hp16 than Hc due to bacterial degradation of the molecular structures of heart proteins. Greater amplitude of amide II bands of 2Hh16 and 2Hp16 than Hc suggested greater loss of molecular order of protein in 2Hh16 and 2Hp16 than Hc owing to microbial breakdown of protein molecules in treated samples than control Hc. The SEM micrographs revealed that both 2Hh16 and 2Hp16 had smaller particle size, surface cracking and rough texture in comparison to Hc as shown in figure 1b.

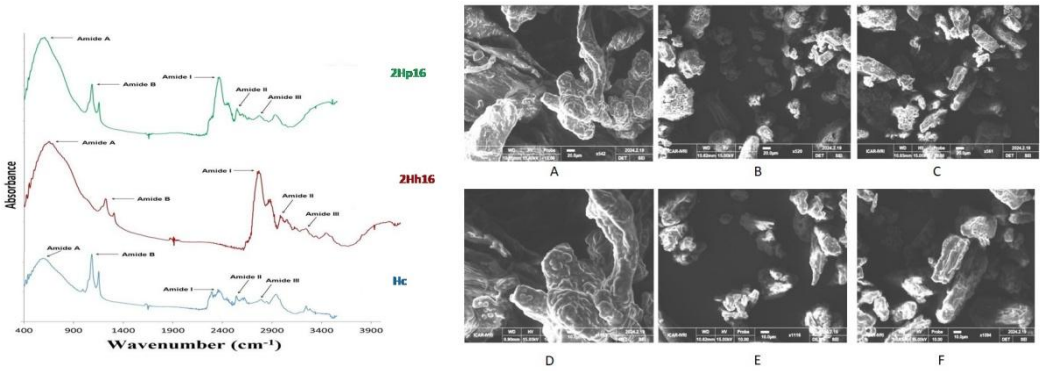

Fig. 1: (a) FTIR spectrum of Hc- Freeze-dried raw heart; 2Hh16- 2% L. helveticus, 16 h; 2Hp16- 2% L. plantarum, 16 h. Fig. 1. (b). Scanning electron micrographs of freeze-dried raw heart, Hc (A: x500, D: x1000), freeze-dried heart hydrolysate fermented with L. helveticus, 2Hh16 (B: x500, E: x1000) and freeze-dried heart hydrolysate fermented with L. plantarum, 2Hp16 (C: x500, F: x1000).

IV. CONCLUSION

This study examined the characteristics of chicken heart hydrolysates produced through bacterial fermentation using *L. helveticus* and *L. plantarum*. The analysis demonstrated that the physico-chemical properties of the hydrolysates were influenced by the specific bacterial culture used. Thus, fermentation process with Lactobacillus bacteria presents a promising method for generating protein hydrolysates from chicken heart. These findings suggest potential applications for incorporating chicken heart hydrolysate obtained by fermentation process with LAB into novel functional food products.

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ENHANCING BOVINE SATELLITE CELL GROWTH WITH CHITOSAN-MODIFIED MICROCRYSTALLINE CELLULOSE SCAFFOLDS

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I. INTRODUCTION

In recent years, there has been a burgeoning interest in developing cellulose structures with enhanced characteristics for biomedical applications [1,2]. These structures are particularly sought after for their biodegradability, biocompatibility, and extensive surface area. Cellulose's ability to support high cell density and modulate pore size, along with its capability to structure in both random and aligned fiber arrangements, has positioned it as a favorable scaffold material for cultivated meat production [3]. While previous studies have largely focused on cellulose derivatives, our research aims to harness the potential of pure cellulose by utilizing electrospun nanofiber matrices for muscle cell cultivation, leveraging their structural benefits for improved cell proliferation.

II. MATERIALS AND METHODS

2.1. Development of electrospun microcrystalline cellulose scaffold

2.1.1. Electrospun microcrystalline cellulose scaffold

Microcrystalline cellulose was dissolved in a 1:1 ratio of 1-butyl-3-methylimidazolium acetate (an ionic liquid) and dimethyl sulfoxide (co-solvent) and then electrospun using a wet-type apparatus to form a smooth nanofibrous scaffold (eMCS). The eMCS underwent a three-stage elution with deionized water and ethanol for purity [4], followed by lyophilization to enhance its porosity and surface-area-to-volume ratio.

2.1.2. Chitosan-modified electrospun microcrystalline cellulose scaffold

Surface modifications were applied to the eMSC (2.1.1.) to create the chitosan-modified scaffold. The eMCS was immersed for 1-3 hours in a 0.02 g/ml chitosan solution prepared using 2% wt. acetic acid solution to produce a chitosan-modified electrospun microcrystalline cellulose scaffold (eMCSch).

2.2. Testing of the electrospun microcrystalline cellulose scaffolds for primary bovine satellite cell proliferation

2.2.1. Bovine satellite cell proliferation

Bovine Satellite Cells (BSCs) were isolated from a one-month-old Holstein bull calf weighing 59 kg, sourced from Aarhus University Cattle facility (DKC, 8830 Tjele). The M. semimembranosus muscle was then excised from the carcass and transported on ice to the Cell Laboratory at the Department of Food Science, Aarhus University. Cell isolation commenced approximately 2 hours postmortem, following the protocol established by Skrivergaard et al. (2021) [5]. Isolated BSCs were resuspended in 37°C growth media (GM) consisting of Dulbecco's Modified Eagle Medium (1X) + GlutaMAXTM (DMEM) (61965-026, Gibco) supplemented with 1% Pen Strep (1:1) (15140122, Gibco), 0.2% Gentamicin sulfate (L0012100, Biowest), 1% Amphotericin B (15290026, Gibco), 1% sodium pyruvate (11360-070, Gibco), 10% FBS (10082147, Gibco), and 10% HS (26050-088, Gibco). The cell solutions were seeded on the cellulose matrix with cell densities of 5,000-50,000 cells per well in cell repellent 96-well plates (174925, Thermo Scientific). The seeded BSCs were cultured for three days at 37°C in a 5% CO_2 humidified atmosphere.

2.2.2. Bovine satellite cell viability

The viability of BSCs cultured on the eMCS and eMCSch was assessed using a metabolic assay with Cell proliferation Reagent WST-1 (11644807001, Roche). The WST-1 reagent was added in a 1:10 ratio and incubated at 37°C in a 5% CO_2 humidified atmosphere for 1h. The assay measured the absorbance at a wavelength of 440 nm for formazan and 650 nm for reference using Cytation 5 Cell Imaging Reader (BioTek), which indicates relative cell viability.

III. RESULTS AND DISCUSSION

3.1. Development of the electrospun microcrystalline cellulose scaffolds

Research determined that the optimal cellulose concentration for electrospinning microcrystalline cellulose scaffolds (eMCS) is 10% wt, with a solvent/co-solvent ratio of 1:1. Under these conditions, the resulting eMCS exhibited a smooth structure and consisted of cylindrical fibers. The optimal conditions for wet-type electrospinning were found to be a flow rate of 6.28 ml/h, a voltage of 10-13 kV, and a distance of 2 cm from the 22-gauge steel needle to the collector.

The research on the development of a chitosan-modified scaffold (eMCSch) determined that optimal parameters for the chitosan solution used in the modification process were determined to be a concentration of 0.02 g/mL in 2% acetic acid, with the eMCS scaffold immersion time of 3 hours. This concentration effectively ensured complete coverage of the eMCS surface and allowed penetration into the deeper layers of the eMCS without compromising its inherent fibrous structure.

3.2. Testing of the electrospun microcrystalline cellulose scaffolds for primary bovine satellite cell proliferation

The efficacy of both the eMCS and eMCSch scaffolds in supporting primary bovine satellite cell (BSC) proliferation was assessed. The chitosan-modified scaffold (eMCSch) significantly enhanced cell viability, showing a corrected absorbance increase to 0.090 ± 0.022, compared to the unmodified scaffold (eMCS), which recorded an absorbance of 0.048 ± 0.009. This data indicates an improved cellular environment due to the chitosan modification.

While initial cell adhesion and proliferation were observable on the eMCSch, the presence of clearly defined cells was limited. Neither scaffold exhibited significant cytotoxic effects, as evidenced by cell counts in the medium with scaffold residues, which were comparable to control values. These findings underscore the potential of chitosan-modified scaffolds in tissue engineering, although further optimization may be necessary to enhance cellular integration and proliferation.

IV. CONCLUSION

This study has demonstrated the potential of chitosan-modified microcrystalline cellulose scaffolds (eMCSch) to enhance the initial viability and growth of primary bovine satellite cells (BSCs). The findings revealed that the eMCSch significantly improved cell viability, as evidenced by the increase in corrected absorbance measurements compared to the unmodified electrospun microcrystalline cellulose scaffold (eMCS). These results underscore the effectiveness of chitosan modifications in creating a more conducive cellular environment for tissue engineering applications.

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FRESH SAUSAGE ADDED ENCAPSULATED AÇAÍ OIL AS ANTIOXIDANT POTENTIAL

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I. INTRODUCTION

The antioxidant capacity of the açaí fruit and its derivatives has generated significant interest, particularly its oil and potential role as a food or food ingredient [1]. Microencapsulation techniques have been widely used, particularly in compounds with bioactive properties, allowing controlled release under specific conditions [2]. Thus, the aim of the study was to use açaí oil capsules with antioxidant potential in fresh sausages frozen for 90 days and to evaluate the oxidative and sensory characteristics.

II. MATERIALS AND METHODS

The açaí oil was encapsulated through the ionic gelation process using sodium alginate in order to control the release of acai oil during the storage period. The fresh sausages were prepared using ground pork, pork backfat and spices and were filled in natural casing. Four treatments were developed: control (C) without the addition of açaí oil capsule; T1 with 1.5g/kg of açaí oil capsule; T2 with 2.5g/kg of açaí oil capsule and T3 with 3.5g/kg of açaí oil capsule. The pH was determined by the Hanna pH meter (Hanna Instruments, Woonsocket, USA). The determination of substances reactive to thiobarbituric acid (TBARS) was carried out according to Raharjo et al. [3] and protein oxidation was analyzed according to the method described by Levine et al. [4]. The sensory analysis of the sausages was carried out using an acceptance test with 10 pre-trained panelists aged 22-47, members of the meat laboratory and familiar with the product. All analyses were carried out in triplicate and analyzed using ANOVA and the Tukey test through Statistica® 7.0 software with a 5% significance level.

III. RESULTS AND DISCUSSION

The pH average between 5.64 and 5.72 on day 0 and 5.12 to 5.43 on day 90, with the samples with capsules showing higher values than the control ($P < 0.05$). A possible explanation is that the alginate used to form the capsules contributed to increased pH values, as it has a basic pH. Regarding storage time, there was a decrease in pH values for all treatments ($P < 0.05$).

In relation to TBARS values the values averaged between 0.22 on day 0 and 0.96 mg of MDA/kg on day 90 of storage. Açaí oil capsules were not as effective in inhibiting lipid oxidation. In future work, it is suggested to increase the concentration of encapsulated oil added.

In the protein oxidation results (Figure 1), it can be seen that on day 0, T2 showed the highest levels of oxidation. On day 90, the control and T2 showed the highest levels. In other words, the release of oil from the capsules was not homogeneous, with T1 having a lower açaí content than T2, and even so it showed more promising results.

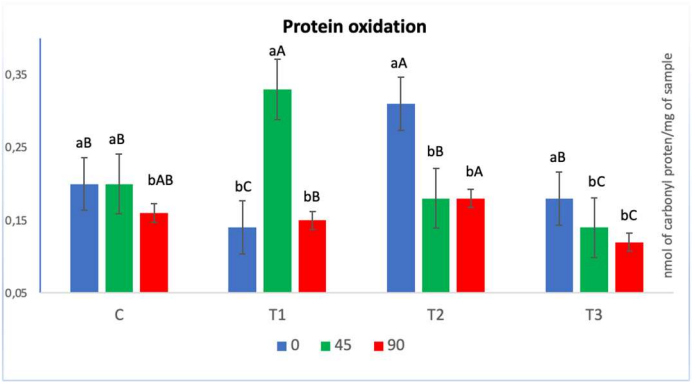

Figure 1. Protein oxidation of fresh sausages.
a-b Mean values with different letters showed significant differences between treatments (P < 0.05) by Tukey's test. A-C Mean values with different letters showed significant differences between days (P < 0.05) by Tukey's test.
Control treatment – C: without inclusion of açaí oil capsules; T1: inclusion of 1.5 g of açaí oil capsules / kg of sausage; T2: inclusion of 2.5 g of açaí oil capsules / kg of sausage; T3: inclusion of 3.5 g of açaí oil capsules /kg of sausage.

There was no significant difference (P > 0.05) between treatments for the sensory attributes global acceptance, color, aroma, flavor, and texture. According to Meeilgard et al. [5], panelists "moderately liked" the color, aroma, texture and flavor. A score of 7.94 was obtained for the global acceptance, indicating that overall, they "moderately liked" the product. Regarding purchase intention, there was no significant difference (P > 0.05) demonstrating that the panelists "maybe bought, maybe not."
The scores of all evaluated attributes did not vary significantly between treatments, including the control treatment. This suggests that the addition of açaí oil may be a promising alternative, as in addition to improving the fatty acid profile and containing antioxidant properties, it does not alter the sensorial quality of the products. This is particularly exciting from a nutritional standpoint as açaí oil is a known rich source of essential fatty acids, antioxidants, and other health-beneficial nutrients [6].

IV. CONCLUSION

Açaí oil capsules and storage period influenced the pH of fresh sausages. Açaí oil was not very efficient in inhibiting lipid oxidation and protein. The sausage samples with the capsules were well accepted sensorially, suggesting that it is possible to develop fresh sausages with the addition of açaí oil.

ACKNOWLEDGEMENTS

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INVESTIGATING THE EFFECTS OF LOW-SALT PROCESSING ON THE UMAMI PEPTIDES OF DRY-CURED HAM USING PEPTIDOMICS TECHNIQUES

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I. INTRODUCTION

Salt contributes to the hydrolysis of muscle proteins in dry-cured hams, forming peptides and other small molecules that impart sweet, sour, salty, fresh, and bitter flavors, enriching and improving the sensory characteristics of the hams. The salt reduction can change the complex protein hydrolysis system, causing product variations [1]. The aim of this study was to compare the differences in umami peptides between low- and full-salt dry-cured hams using peptidomics.

II. MATERIALS AND METHODS

2.1 Dry-cured ham preparation

Dry-cured hams were processed as previously described [2]. Briefly, 24 bone-in fresh hind legs (10-12 kg for each) from Landrace pigs were purchased from a local slaughterhouse (Yanji, China) and they were equally assigned to two groups. The low-salt group was cured using a mixture of 70% NaCl, 18% potassium lactate, and 12% lysine, and the full-salt hams were cured with 100% NaCl. The amount of curing agent was 6% of each leg weight. *Biceps femoris* (BF) muscles from two groups at the end of processing were collected in marked aseptic bags and stored at -80 ℃.

2.2 Qualitative and relative quantitative analysis of peptides using peptidomics

Three samples were randomly selected from each group to extract peptides, and the method was referred to Zhang et al. [3]. Peptides were separated by the EASY-nLC 1000 ultra-high pressure liquid chromatography and then analyzed by a Q-Exactive mass spectrometer.

2.3 Identification of umami peptides

Peptide sequences were imported into the iUmami-SCM (https://camt.pythonanywhere.com/iUmami-SCM) to predict umami peptides in the low- and full-salt groups [4].

2.4 Data analysis

Orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed using the software SIMCA version 14.1.0 (Umetrics, Umea, Sweden), and the significant significance was set as $P < 0.05$. Significantly different umami peptides (SDUPs) between the two groups were identified using variable importance in projection (VIP > 1.0) based on the weighted sum of the squares of the OPLS-DA.

III. RESULTS AND DISCUSSION

According to Figure 1A, 2,302 and 1,262 peptides were identified below 3 kDa in the low- and full-salt groups, respectively, among which 968 peptides were shared in both groups. According to the prediction results from the iUmami-SCM website, 633 umami peptides were shared by both low-salt hams (1,524 umami peptides) and full-salt hams (818 umami peptides). Besides, 62.72% (397/633) of the shared umami peptides showed significant changes in relative abundance. Specifically, the relative abundances of 168 and 229 umami peptides were significantly up- and down-regulated (fold change > 1.5 and $p < 0.05$) in the low-salt group compared with the full-salt group (Figure 1B). The OPLS-DA model was developed to discover characteristic umami peptides (Figure 1C). Finally, a total of 1,011 SDUPs were screened. Table 1 lists the protein sources of umami peptides with VIP score > 1.0 (Top 5). The difference in the relative abundance of umami peptides in low- and full-salt dry-cured

hams was significantly affected by the hydrolysis of proteins such as creatine kinase M-type (CKM), fast skeletal muscle troponin T (TnTf) and myosin-1.

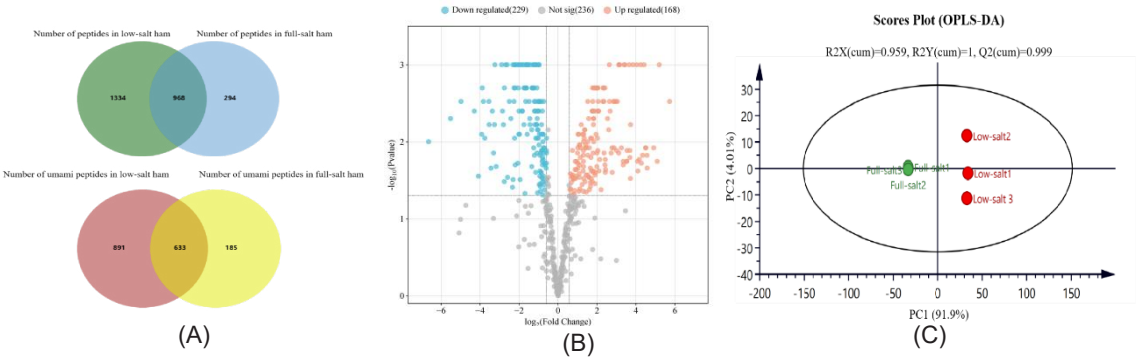

Figure 1. (A) Amounts of peptides and umami peptides in low- and full-salt dry-cured hams; (B) Volcano map; (C) OPLS-DA score plot: PC1 = 91.9%, PC2 = 4.01%, R2X(cum) = 0.959, R2Y(cum) = 1, Q2 = 0.999.

Table 1 – Precursor protein of the SDUPs with VIP score > 1.0 between the low- and full-salt groups.

Number	Protein names	Gene Names	Total VIP>1 SDUPs	Low-salt SDUPs	Full-salt SDUPs
1	Creatine kinase M-type	CKM	163	140	66
2	Fast skeletal muscle troponin T	TNNT3	149	147	23
3	Myosin-1	MYH1	113	90	53
4	Myosin light chain 1	MYL1	74	65	25
5	Phosphopyruvate hydratase	ENO3	63	54	13

Only up to five precursor proteins were shown.

IV. CONCLUSION

Overall, low-salt processing altered the umami peptide profiles of dry-cured hams, producing more unique umami peptides. There were 1,011 SDUPs in the low- and full-salt groups, which accounted for 59.36% of the total umami peptides. Meanwhile, 36.18% and 26.54% of the shared umami peptide abundance was down- and up-regulated in the low-salt group compared to the full-salt group. The SDUPs were mainly derived from differential hydrolysis of CKM, TnTf, and myosin-1. This study offers preliminary evidence for investigating flavor alterations in low-salt dry-cured hams.

ACKNOWLEDGEMENTS

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SESSION 10

Meat Products Stability

Thursday 22 August 2024

Analysis of the temperature stability of essential oils and their effects when applied to fermented sausages

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I. INTRODUCTION

Maintaining consistent temperature and humidity in the fermentation chamber is crucial for optimal growth of starter fungi in fermented sausages. Fungal species like Cladosporium cladosporioides can negatively affect sausage quality by producing off-flavors, color changes, and hairy mycelium. Essential oils, with antibacterial and antifungal properties, enhance food safety. This study evaluates the antifungal activity of clove, marjoram, basil, black pepper, and rosemary essential oils against common molds in fermented sausages to identify the most effective natural mold reducer during production.

II. MATERIALS AND METHODS

Temperature resistance testing was conducted at 4, 10, 15, and 20°C to assess the stability of essential oils used in fermented sausage production. The oils were exposed for 2 hours at each temperature, then applied to paper discs inoculated with fungal spores. Inhibition zones were measured after culturing at 25°C for 3-5 days. For making fermented sausage, three methods are used: i) addition of dry clove powder to the dough at 20 g/kg, ii) spraying clove extract onto the sausage surface (about 5 ml), and iii) immersing sausages in 2 liters of clove extract solution for up to 1 minute.

III. RESULTS AND DISCUSSION

Clove consistently exhibited inhibitory effects averaging over 36 mm, regardless of temperature. Another study showed clove oil had significant inhibitory effects over 40 mm against P. oxalicum, P. commune, and C. cladosporioides, also unaffected by temperature. Overall, essential oils showed no significant differences with temperature ($P > 0.05$). Regression analysis indicated very low correlation (r-square 0.00 to 0.12), suggesting their efficacy is preserved during fermentation, refrigeration, and consumption, making them suitable for application.

IV. CONCLUSION

Clove shows strong potential for inhibiting Penicillium spp. and Cladosporium cladosporioides in fermented sausages. While clove ethanol extract has lower antibacterial effects than clove oil, it is easier to apply in small-scale farms. Temperature stability analysis confirmed consistent antifungal ability at 4, 10, 15, and 20°C, with a low correlation in regression analysis (r-square < 0.12). Both spraying and dipping treatments reduced general bacteria by 1.2 log CFU/g and fungi by 0.3 log CFU/g. Considering cost, worker convenience, antimicrobial effects, and quality, spraying treatment is recommended for manufacturing.

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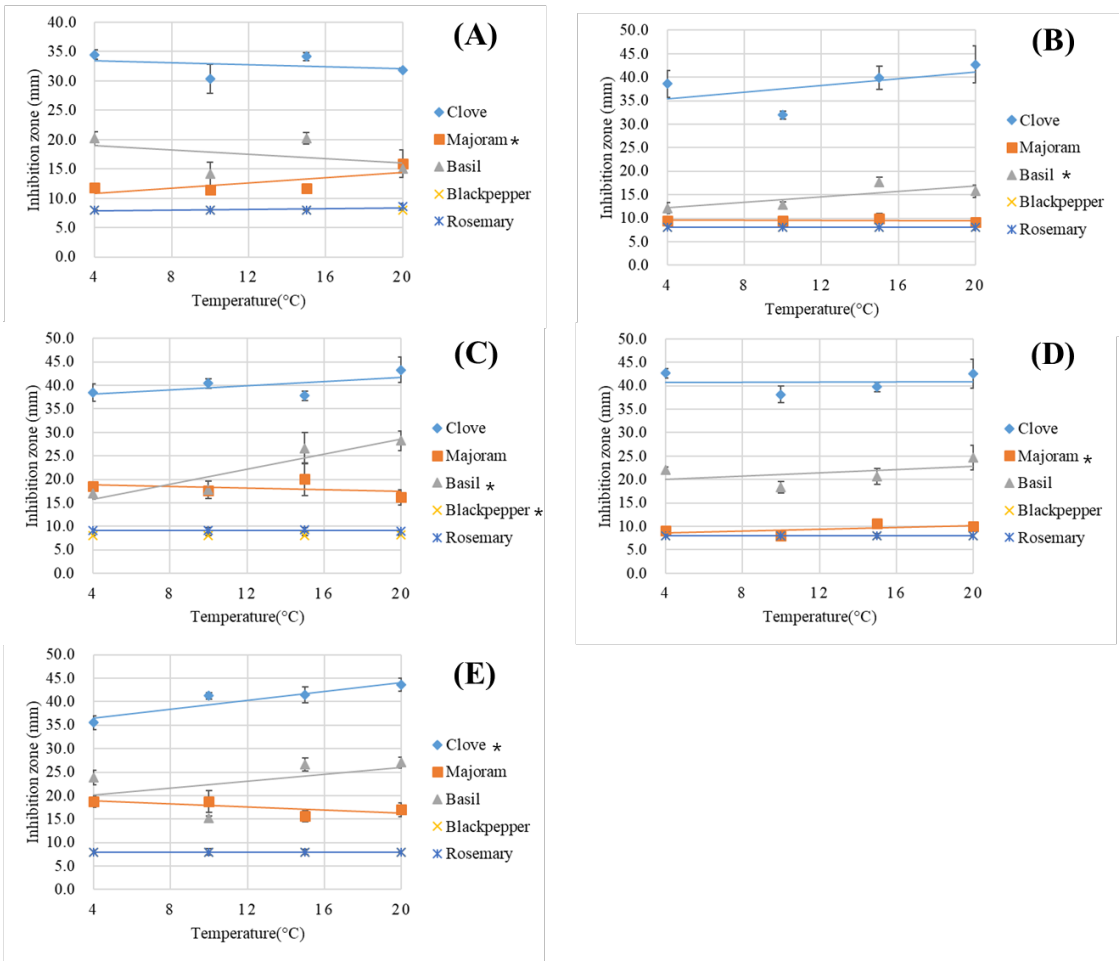

Figure 1. Analysis of antifungal activity according to temperature resistance of essential oils. * indicate that temperature has a significant ($P < 0.05$) effect on antifungal inhibition of essential (A) *P. commune*, (B) *P. chrysogenum*, (C) *C. cladosporioides*, (D) *P. oxalicum*, (E) *P. solitum*

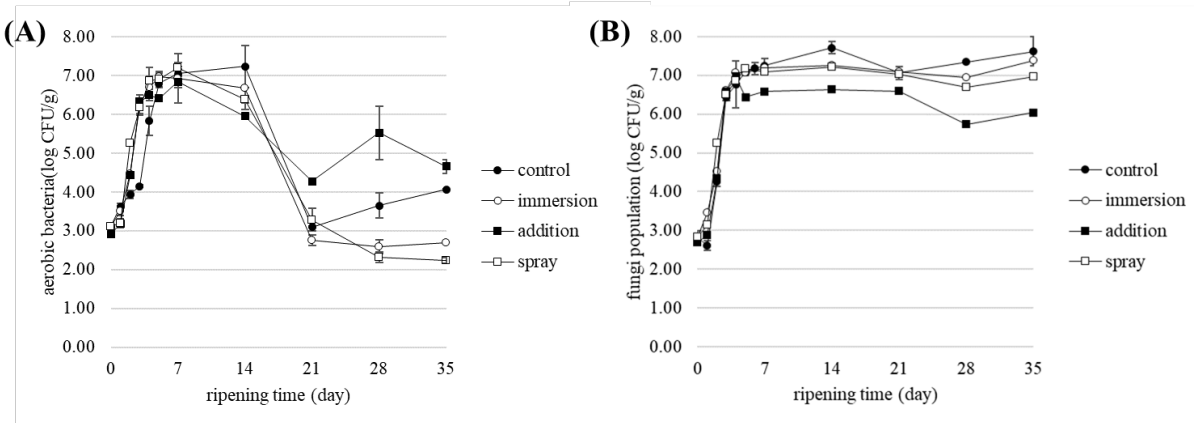

Figure 2. Changes in aerobic bacteria and fungi count during the ripening period of fermented sausages according to clove treatment methods.

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PUMPKIN FLOWER AS A NATURAL ANTIOXIDANT IN CHICKEN PATTIES

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I. INTRODUCTION

One of the main deterioration processes in meat that impacts its nutritional value, and sensory properties is lipid oxidation [1]. Maintaining lipid stability often requires the incorporation of antioxidant compounds. However, their use has been limited by international organizations like the U.S. FDA and the WHO due to several safety concerns about their presence in foods [1,2]. Consequently, there is a growing interest in obtaining antioxidants from natural sources. Recently, pumpkin flower (*Cucurbita maxima*) has emerged as a potential source of interesting biocompounds. These flowers are abundant in minerals, polyunsaturated fatty acids, and antioxidants. In this study the antioxidant effect of pumpkin flower powder (*Cucurbita maxima*) dehydrated by foam-mat drying, freeze drying and oven drying was evaluated in chicken patties.

II. MATERIALS AND METHODS

Three different drying methods were evaluated to obtain pumpkin flower powders. These methods included foam-mat drying (FF, CF), freeze drying (LF), and oven drying (OF) [3]. In foam-mat drying, the edible flowers were converted into a stable foam by using albumin maltodextrin, hydroxyethyl cellulose, and Tween-80 as foaming agents and stabilizers, and then dried by application of hot air. Four formulations of chicken patties composed by chicken breast meat, NaCl, binding protein, and pumpkin flower were designed. FF and CF batches included 1.5% of the flower powder, LF and OF additives consisted only of the dried pumpkin flower (0.05%). A control sample without pumpkin flower was also considered. All samples were vacuum packaged and stored at 4 °C for 7 days [4]. Antioxidant assays and color evaluation were performed on days 0 and 7, before and after cooking at 69 °C internal temperature in a microwave. The evaluation of the antioxidant profile of the chicken patty samples was carried out by DPPH, ABTS, and FRAP methodologies [5]. Lipid oxidation was evaluated following the development of thiobarbituric acid reactive substances (TBARS) [6]. Moisture content in chicken patties was measured and CIELa*b* parameters were determined.

Sensory evaluation of the cooked patties was performed using a 5-point hedonic test conducted by fifteen trained panelists. Hedonic scores ranged from 1 to 5 representing from very unpleasant (1) to excellent (5). The test included the evaluation of color, odor, texture, taste, and overall acceptability [4, 7]. Statistical analysis of the data obtained was performed using Minitab 17 software. Mean values were compared using the one-way ANOVA and Tukey multiple range tests were used to estimate the level of significance among chicken patties. Principal component analysis (PCA) was performed to outline differences and groupings among samples.

III. RESULTS AND DISCUSSION

Four formulations were developed to be evaluated as chicken patties additives to provide color and increase antioxidant activity in the final product (Figure 1). In fresh chicken patties a significant improvement of the antioxidant properties was observed with the incorporation of the pumpkin flower additives. However, this antioxidant effect was affected by cooking process. Nevertheless, the formulations containing the additives continued presenting higher scores compared to the control samples. After cold storage, this trend remained.

Figure 1. Pumpkin flower powders.

The FF, CF, and LF formulations exhibited the higher antioxidant activity compared to the samples with oven dried pumpkin flowers. The addition of the flower additives in the formulation prevented the oxidation process of lipids determined by TBARs during storage and cooking compared with the control samples. According to the results obtained in the analysis of antioxidants, the drying process of the powder played an important role in the preservation of bioactive compounds. The foam-mat drying method allowed the preservation of bioactive compounds after cooking and after cold storage compared with the conventional drying method due to the encapsulation-like mechanisms of the added proteins. Sensory evaluation of the chicken patties was carried out on the cooked patties on day 0 and day 7 of storage. On the first day, hardly any difference between samples was found. The panelists gave the highest score to CF formulation with an overall acceptability of 4.62. For odor, FF, CF, and LF formulations presented the most attractive aroma to panelists. After 7 days of storage (4°C) no changes in color were appreciated. Related to odor, no unpleasant odors were perceived in CF and LF patties. In evaluation of taste, a similar pattern was observed.

IV. CONCLUSION

This study pioneers the use of pumpkin flower (*Cucurbita maxima*) as an antioxidant additive in chicken patties. Assessment through DPPH, ABTS, and FRAP assays showed the effectiveness of pumpkin flower in reducing oxidation during cooking and storage, enhancing sensory qualities. Foam-mat and freeze-drying methods exhibit superior antioxidant and sensory effects compared to oven-drying. Antimicrobial activity can be inferred by control and OF samples' higher spoilage. Foam-mat drying emerged as the preferred method to incorporate fresh pumpkin flowers into chicken patties, given its simplicity, efficiency, and cost-effectiveness.

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EFFECT OF "CHORIZO" FORMULATION (CLEAN LABEL *VS.* TRADITIONAL) ON THE CHEMICAL COMPOSITION, COLOR AND LIPID STABILITY DURING THE STORAGE PERIOD

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I. INTRODUCTION

The current trend in the meat industry is the development of differentiated high-quality products. In this regard, the Celta breed presents differentiated characteristics and is highly appreciated by the consumer. However, consumers are increasingly concerned about the diet-health relationship, prefer to consume more "natural" products, and reject products with synthetic additives. Thus, the meat industry has to take a radical turn and adapt to the demands of the market. Therefore, this study aimed to develop a 100% Celta pig chorizo (clean label) and to evaluate the reformulation effect on the composition, color, and oxidative characteristics over 6 months of self-life.

II. MATERIALS AND METHODS

Two batches of chorizo were made, the control batch with additives (ascorbic acid, sodium phosphate, potassium nitrate, sodium nitrite, lactose, and soy protein), and the clean label samples with a commercial preparation based on natural extracts (Ligavi Sine ST) provided by ANVISA (Madrid, Spain; www.anvisa.com). Both were made with the same traditional recipe. The meat mass was composed of shoulder (55%), lean (25%), and bacon (20%), and the seasonings were: sweet paprika (18 g/kg), garlic (10 g/kg), hot paprika (3 g/kg) and oregano (0.5 g/kg). After stuffing, the chorizos were smoked and cured at 14 ºC and 75% HR for 21 days. After this period, they were vacuum packed in Pa/Pe film (90 µm; oxygen permeability <60 $cm^3/(m^2*24h*atm)$ and kept refrigerated (2ºC) under dark conditions. Chemical composition (after curing), color evolution, and oxidative stability during the self-life (0, 2, 4, and 6 months of storage) were evaluated. Moisture, ash, and proteins were determined following international ISO procedures, and fat was calculated following the AOCS procedure. Color parameters were measured using a portable colorimeter (CR-600d, Minolta Co. Ltd., Osaka, Japan), while lipid oxidation was determined by measuring the TBARs index using the Vyncke [1] procedure. The statistical analysis of the data (ANOVA) was carried out using SPSS software (version 25).

III. RESULTS AND DISCUSSION

The composition parameters (Table 1) showed that the moisture (20.6 g/100 g), fat (~50 g/100 g), and protein (23.5 g/100 g) were not influenced by the reformulation. However, Control samples had the highest ash content (p<0.001) (3.09 *vs*. 2.72 g/100 g), which could be due to the preparations used in the formulation of both types of chorizos. Our results agree with previous studies carried out in Celta Pig chorizo [2]. In meat products, color is a vital factor, since it will determine the consumer's purchase intention. This is why any reformulation must maintain the typical color of the traditional product since it affects its acceptability. The values of L* (~43), a* (~20) and b* (~18) after curing showed no differences due to the reformulation, and coincided with those previously described in chorizos [3,4]. Color parameters did not show significant differences (except slight changes in L*) between batches during the 6-month shelf-life.

Concerning oxidative stability (Figure 1), the TBARs value was significantly higher in the clean label chorizos after curing (0.47 *vs*. 0.38 mg MDA/kg), a value that was equal to the control chorizos in the second month of storage, and it was even lower in months 4 and 6 of storage. The

values varied between 0.27 and 0.38 mg MDA/kg in the control chorizos and between 0.25 and 0.47 mg MDA/kg in the clean label ones. Similar oxidation values were described by other authors in chorizo [2,4].

Table 1 – Chemical composition and color parameters of chorizo after curing

	Control	Clean label	*p*-Value
Composition (g/100 g)			
Moisture	20.8±1.14	20.4±1.90	0.718
Fat	50.0±1.29	49.6±2.21	0.739
Protein	22.7±1.11	24,0±0.87	0.087
Ash	3.09±0.10	2.72±0.08	<0.001
Color parameters			
L*	45.1±2.94	43.2±1.07	0.205
a*	19.5±3.54	21.4±1.16	0.268
b*	17.4±3.76	19.4±2.51	0.352

The decrease in TBARs during the shelf life is due to the absence of oxygen in the packaging and the presence of antioxidants in the sausages, which cause the free radicals to be neutralized, promoting the decrease or stabilization of oxidation when packaging products under vacuum. In any case, the TBARs values observed in this study, in both the control and clean label chorizos, are well below the limits of the sensory perception threshold of rancid odors or flavors.

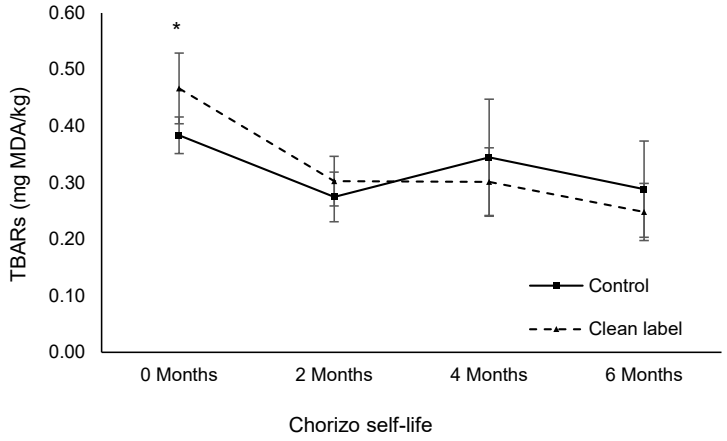

Figure 1. Evolution of lipid oxidation in chorizo over the 6 months of shelf-life (2ºC vacuum packed). *P<0.05.

IV. CONCLUSION

The reformulation strategy proposed in this study demonstrates that the production of a clean label chorizo is viable, without negatively affecting either the composition or the evolution of their quality during shelf-life. Furthermore, the development and production of these differentiated products with great acceptance by the consumer is crucial to improving the competitiveness of producers.

ACKNOWLEDGEMENTS

This study was supported by the project 2022/001A from “Rural Development Program (PDR) of Galicia 2014-2020” and financed with FEADER funds.

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PRICKLY PEAR ENCAPSULATED EXTRACT AS ANTIOXIDANT IN BEEF PATTIES

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I. INTRODUCTION

In the meat industry, additives are essential due to the high-water activity of meat, its grinding process and raw presentation, which facilitates microbial growth and accelerates oxidation reactions. The prickly pear (*Opuntia ficus-indica*) is the fruit of the prickly pear, which is rich in betalains, a water-soluble nitrogenous pigment, which includes red violet betacyanins and orange yellow betaxanthins [1]. In addition to their colouring capacity, betalains show antioxidant activity [2]. Therefore, obtaining powder extracts rich in betalains derived from the skin of the prickly pear can promote its valorization and the inclusion of natural additives in the meat industry. The present work aimed to evaluate the antioxidant ability of prickly pear encapsulated extract in beef patties after 9 days of cold storage.

II. MATERIALS AND METHODS

Ultrasonicator UP400St a 180 W and 24kHz accoupled with S24d22D probe was employed to extract betalains from the prickly pear (*Opuntia ficus-indica*) peel with the following extraction conditions: time: 6 minutes; extractant: EtOH (40%, pH 3.5), solid to solvent ratio 1:35; amplitude 100%. After evaporating EtOH, the betalain-rich extract was dehydrated using a spray-dryer (Mini SprayDryer B-290; Büchi) with maltodextrin as a vehicle (11.5 g/100 mL extract Four batches of 2 kg each, whose common ingredients were beef, water, and salt, were made. The baches were negative control [N-CON.] (without additives), positive control [P-CON.] (with the addition of sodium erythorbate at 0.5 g/kg), and two formulations with the addition of 2.5 and 5.0 g/kg of Prickly pear encapsulated extract (PPEE) [PP-2.5] and [PP-5.0]. The chemical composition and the oxidations in raw beef patties stored at 4ºC. were evaluated. The chemical composition was achieved following the corresponding AOAC method. The lipid oxidation was evaluated with the thiobarbituric acid reactive substances (TBARS) assay. Protein oxidation was determined following DNPH method. Significant differences were determined by means of one-way ANOVA and two-way ANOVA (day and treatment).

III. RESULTS AND DISCUSSION

The inclusion of synthetic additive, sodium erythorbate, and prickly pear encapsulated extract in the beef patties formulations did not modify beef patties chemical composition and physicochemical properties as can be expected (Table 1) due to the low amount of both substances added to the beef patties. The rancidity threshold perceptible by consumers is set between 1.5 – 2.0 mg malonaldehyde (MDA)/kg. [3] As can be seen in Figure 1A, the N-CON patties on day 9 presented rancidity values perceptible by consumers (1.75 mg MDA/kg). On the other hand, both patties added with PPEE did not reach values greater than 1.37 mg MDA/kg on day 9. Furthermore, both tested concentrations were effective to slow down significantly lipid oxidation (p <0.05). Concerning to protein oxidation, PP-5.0 showed lower protein oxidation values than the rest of the batches analyzed at day 9. However not significant differences were detected among the four studied batches.

Table 1. Chemical composition and physicochemical properties of beef patties at day 0.

	N-CON	P-CON	PP-2.5	PP-5.0
Moisture	73.25 ± 0.38	73.79 ± 0.29	72.97 ± 0.67	73.21 ± 0.55
Fat	6.36 ± 0.53	5.85 ± 0.47	6.48 ± 0.58	6.58 ± 0.62
Protein	18.29 ± 1.06	18.34 ± 0.82	18.19 ± 0.14	17.89 ± 0.36
Ash	1.85 ± 0.09^{ab}	1.80 ± 0.02^{a}	1.97 ± 0.04^{a}	1.94 ± 0.02^{b}

Different letters in the same row indicate significant differences among formula. Statistically significant differences were considered when p <0.05 after Tukey`s post hot test.

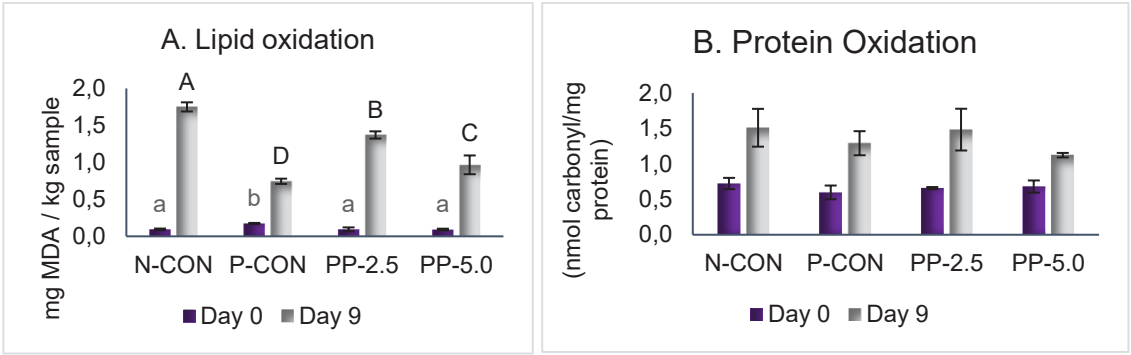

Figure 1. A. Lipid oxidation and B. protein oxidation of four studied baches of beef patties on days 0 and 9 of cold storage. Different letters indicate significant differences among treatments. Statistically significant differences were considered when p <0.05 after Tukey`s post hot test.

IV. CONCLUSION

Prickly pear encapsulated extract presented potential to be a natural antioxidant for meat industry, especially for mitigate lipid oxidation. Between the two concentrations studied, the highest concentration (5 mg/kg) was more effective in reducing lipid oxidation. During the 9 days of storage, the beef patties are preserved without presenting a perceptible level of rancidity. However, protein oxidation didn't show significant changes during storage.

ACKNOWLEDGEMENTS

This study was supported by the Spanish Ministry of Science and Innovation project (PID2021-123628OB-C41-Agri-food co-products as a source of bioactive compounds for the development of functional meat products), which is funded by MCIN/AEI/10.13039/501100011033/ and FEDER, UE. The work of Laura Moraga Babiano was supported in part by MCIN/AEI/10.13039/501100011033 and in part by the FSE+ under the grant PRE2022-105858. Raquel Lucas-González would like to thank the Spanish Ministerio de Universidades for her 'Margarita Salas Requalification' postdoctoral fellowship (funded by the European Union–Next Generation EU).

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PURPLE SWEET POTATO ANTHOCYANINS AS ANTIOXIDANTS AND COLORANT OF PORK PATTIES

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I. INTRODUCTION

In the dynamic landscape of the food industry, green labels have emerged as a beacon of sustainability and health consciousness. In recent years, purple sweet potato (*Ipomoea batata L*) has attracted increasing attention from the food industry. Its popularity has increased, especially in the production of sweet potato chips, in response to the growth in demand and consumption of vegetarian snacks. In addition, the scientific community is exploring various applications of purple sweet potato, such as breakfast cereals, yoghurts, pasta, and cookies, among others [1–3]. The production of these foods generates peel, which, with an economic treatment, could be a source of anthocyanins and a way to valorize food industry co-products. The current work aimed to evaluate the antioxidant and colorant ability of Purple Sweet potato anthocyanins in pork patties after 9 days of cold storage.

II. MATERIALS AND METHODS

Anthocyanins were extracted for dehydrated purple sweet potato peel (*Ipomea batata*) (PSPP). For extraction Ultrasonicator UP400St accoupled with S24d22D probe a 180 W and 24 kHz were used. The following conditions were used for extraction: 5 minutes; extractant: EtOH (60%, pH 3.0), solid to solvent ratio 1:30; amplitude 100%. Then, the rich anthocyanin extract was atomized through a spray-dryer (Mini Spray Dryer B-290; Büchi), using maltodextrin as carried agent (13.5 g/100 g extract). Four patties batches of 2 kg each, whose common ingredients were pork, water, and salt, were made: negative control [N-CON.] (without additives), positive control [P-CON.] (with addition of sodium erythorbate at 0.5 g/kg), and two formulations with addition of 2.5 and 5.0 g/kg of encapsulated anthocyanins from PSPP [A-2.5] and [A-5.0], respectively. The chemical composition and the oxidations in raw pork patties stored at 4ºC were evaluated. Chemical composition was achieved following the corresponding AOAC method. Color was measured in CIELAB space with Minolta spectrophotometer. The lipid oxidation was evaluated with the thiobarbituric acid reactive substances (TBARS) assay. Significant differences were determined by means of one-way ANOVA and two-way ANOVA (day and treatment).

III. RESULTS AND DISCUSSION

The chemical composition of the four-patties treatment was equal, and no significant differences among treatments were observed (Table 1). Anthocyanin extract from PSPP protected lipid oxidation during refrigeration storage, as can be appreciated in Figure 1A. The antioxidant activity against lipid oxidation was better than erythorbate, whose values were higher even than the control (p <0.05). This fact could be due to erythorbate protecting more protein oxidation than lipid oxidation. In addition, the antioxidant ability of anthocyanin extract from PSPP was not concentration-dependent (p <0.05). Therefore, the lowest concentration of anthocyanins extract from PSPP (2.5 g/kg) could be sufficient

to slow down lipid oxidation in pork patties. Concerning color attributes, the action was concentration-dependent and showed only the biggest concentration studied potential as a colorant in pork patties, as can be observed in Figure 1B (p <0.05).

Table 1- Chemical composition of four batches of pork patties.

	Moisture	Fat	Protein	Ash
N-CON	74.14 ± 0.44	5.13 ± 0.53	18.08 ± 0.24	1.83 ± 0.02
P-CON	74.31 ± 0.70	5.35 ± 0.42	17.91 ± 0.22	1.84 ± 0.04
A-2.5	74.75 ± 0.30	4.89 ± 0.18	17.98 ± 0.05	1.83 ± 0.01
A-5.0	73.83 ± 0.33	5.79 ± 0.41	17.86 ± 0.09	1.86 ± 0.01

Different letters in the same row indicate significant differences among formula. Statistically significant differences were considered when p <0.05 after Tukey`s post hot test.

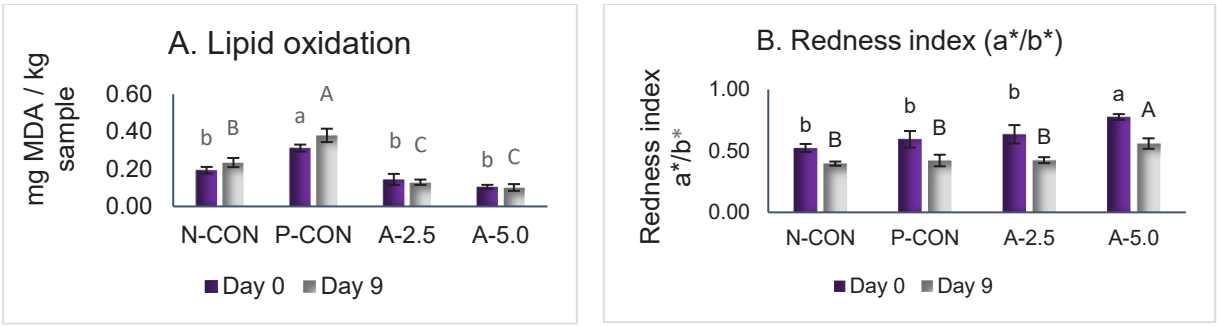

Figure 1. A. Lipid oxidation and B. Redness index values of four studied baches of pork patties on days 0 and 9 of cold storage. Statistically significant differences were considered when p <0.05 after Tukey`s post hot test.

IV. CONCLUSION

Anthocyanin extract from purple sweet potato peel effectively acted as an antioxidant against lipid oxidation in pork patties. Furthermore, at 5 g/kg, the anthocyanin extract presented colorant activity. In conclusion, anthocyanins extract from purple sweet potato peel could be used as a natural additive in the patties elaboration.

ACKNOWLEDGEMENTS

This study was supported by the Spanish Ministry of Science and Innovation project (PID2021-123628OB-C41-Agri-food co-products as a source of bioactive compounds for the development of functional meat products), which is funded by MCIN/AEI/10.13039/501100011033/ and FEDER, UE. The work of Laura Moraga Babiano was supported in part by MCIN/AEI/10.13039/501100011033 and in part by the FSE+ under the grant PRE2022-105858. Raquel Lucas-González would like to thank the Spanish Ministerio de Universidades for her ‘Margarita Salas Requalification’ postdoctoral fellowship (funded by the European Union–Next Generation EU).

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REPLACEMENT SYNTETHIC ANTIOXIDANTS WITH YERBA MATE EXTRACT POWDER IN SALAMI

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I. INTRODUCTION

The use of salts such as nitrite and nitrate in food has been questioned due to the generation of N-nitroso, which are compounds with carcinogenic effects [1]. These salts are intentionally added in the production of most cured meats, such as various types of salami, in order to stabilize color, impart aroma, delay oxidative processes and inhibit the growth of Clostridium botulinum [2]. Natural antioxidants have been studied as substitutes for synthetic antioxidants [3]. Yerba mate (*Ilex paraguariensis*) may be a viable alternative against oxidative processes in meat foods, since it contains phenolic compounds and flavonoids, such as chlorogenic acid and rutin, as well as inhibiting microbial growth [4]. The aim of this study was to use yerba mate extract in salami to assess its capacity as a lipid and protein antioxidant.

II. MATERIALS AND METHODS

SALAMI PRODUCTION

Three salami treatments were developed using pork shoulder meat and pork backfat: Control treatment (Con) - without the addition of nitrite and sodium nitrate; Traditional treatment (Trad) - with the addition of nitrite and sodium nitrate; and treatment with the inclusion of yerba mate extract (ErM), as studied by Pini et al. All the treatments contained starter culture (Flora Italia LC. - CHR HANSEN), salt, sugar, sodium erythorbate and spices. The salami was filled and then fermented and matured (25-18 ºC, RH 85-70%) for 15 days. The shelf life of the products was then assessed for 75 days at 25 ºC.

ANALYSIS OF WATER ACTIVITY (a_w), LIPID AND PROTEIN OXIDATION

Water activity (aw) was analyzed using an Aqualab 4TE (METER Group Inc., Pullman, USA).
Lipid oxidation was determined as described by Raharjo et al [6]. While the concentration of carbonyl groups in the salami was determined using 2,4-dinitrophenylhydrazine, as described by Levine et al.
All the experiments were replicated twice at different times and the analyses were carried out in triplicate.

III. RESULTS AND DISCUSSION

The a_w values showed a significant difference ($p<0.05$) between the treatments only after 45 days of shelf-life; however, the values were typical for salami (average of 0.85) and have been reported by other authors [1].
Regarding lipid oxidation, it can be seen in Figure 1A that the Trad and ErM treatments were different from the control (without nitrite and nitrate) ($p<0.05$) throughout the storage period. On day 75, the treatment with yerba mate was the same as Trad (average 0.55 mg MDA / kg product), so it can be said that the addition of 0.09% yerba mate powder extract was as effective as the addition of nitrite and nitrate.

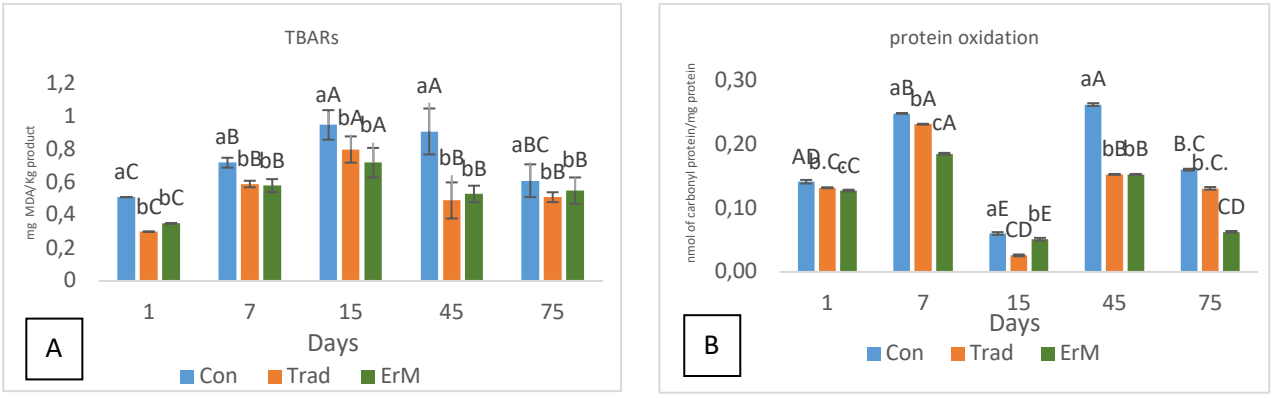

Figure 1: A - Lipid oxidation of salami; B - Protein oxidation of salami. Con – Control Treatment; Trad – Traditional Treatment; ErM – Treatment with the inclusion of Yerba Mate extract. Different lowercase letters differentiate treatments on the same day using the Tukey test ($p<0.05$). Different capital letters differ days in the same treatment using the Tukey test ($p<0.05$).

Figure 1B shows the results of the salami's protein oxidation during the storage period. The values varied, but on most days the Con treatment showed higher levels of protein oxidation, reaching its peak at 45 days of shelf-life. The Trad and ErM treatments behaved similarly, although the oxidation of the ErM treatment was significantly lower than the other treatments. Its oxidation peak at 7 days showed a content of 0.186 nmol of carbonylated protein/mg. At 45 days of storage, the Trad and ErM treatments showed no difference (0.154 nmol of carbonylated protein/mg), and this value was 41.67% lower than the average presented by the Con treatment for the same period.

IV. CONCLUSION

It is concluded that it is possible to replace yerba mate extract in salami, however, additional analyses, such as microbiological ones, should be carried out to test the safety of the products.

ACKNOWLEDGEMENTS

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LIPID AND COLOUR STABILITY OF CURED COW BLOOD SAUSAGE

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I. INTRODUCTION

Blood sausages are ready to eat meat by-products consumed in many parts of the world. The traditional blood sausages contain common salt and are dark brown to black in colour, while modern blood sausages are known for their bright red shiny colour which is obtained by adding nitrite curing salt to the blood [1]. The natural colour of blood sausages comes from the heme pigment, myoglobin. These pigments becomes oxidized and denature causing the red colour to deteriorate or turn brown during storage, which is undesirable to consumers [2]. Hence the objective of this study was to assess the influence of common salt (sodium chloride (NaCl)) and Prague powder (sodium nitrate/nitrite salt) on lipid and colour stability of cow blood sausages over 60 days storage period.

II. MATERIALS AND METHODS

A total of 2 kg (1 kg/ treatment batch x 2 products) of cow blood sausages cured with either common salt (Sausage A) or sodium nitrites salt (Sausage B) were produced in six replicates. The mixture was filled in a synthetic casing. The sausages were cooked in a mini cooker, at an atmospheric temperature of 80 °C to an internal temperature of 75 °C. This was followed by cooling in an iced water bath and kept at 4°C and sampled the following day. All sausages were overwrapped and stored in a retail fridge-type (-10°C) to simulate home freezing. Sausages were evaluated for lipid and colour stability at day 0, 15, 30, 40 and 60 days. Lipid oxidation was assessed by the 2- thiobarbituric acid (TBA) method used by Zereian et al. [3]. Thiobarbituric Acid Reactive Substance (TBARS) values were expressed as malonaldehyde (MA) mg/kg meat product sample. Instrumental colour (L*, a* and b*) of the cooked sausages was measured according to the method described by King et al. [4]. All data were subjected to an analysis of variance (ANOVA) to test for significant treatment effects using GenStat for Windows 22nd Edition [5]. Fisher's protected t-LSD (Least Significant Difference) was calculated to compare means of significant effects at the 5% level. Ethical clearance was obtained for this project: Reference number, AIEC 21/19.

III. RESULTS AND DISCUSSION

Lipid oxidative stability of the cooked cow blood sausages are presented in figure 1. Significant effects on the TBARS of sausages were observed between salt treatments ($P=0.002$) and also over time ($P<0.001$). Sausage A exhibited significantly higher levels of malondialdehyde (MDA) compared to Sausage B, throughout the storage period. This might be due to the pro-oxidant effects of NaCl. Sodium chloride can increase the activity of ionic iron or decrease the activity of antioxidant enzymes [2,6].The lack of antioxidants in NaCl treated sausages allowed catalytic reactions to accelerate over time. The lower TBARS values of Sausage B, containing nitrites, were expected. Nitrite is a typical curing agent and acts against lipid oxidation by binding to heme and preventing the release of the catalytic iron [6]. According to literature, meat products remain non-rancid when MDA levels are under 2-2.5 mg MDA/kg [7]. The sausages analyzed exhibited MDA levels below 2 mg/kg, affirming their non-rancid status. The results for colour parameters, lightness (L*), redness (a*) and yellowness (b*) of the sausages are shown in Table 1. The a* and b* values were significantly affected by treatment

and storage period, respectively. Sausages containing nitrites salt displayed increased redness (P=0.018) compared to sausages containing NaCl throughout the storage period. Greater red colour is an indication of cured meat colour development [1]. The yellowness of both sausages decreased (P=0.031) at day 15 and remained unstable with no significant changes until the end of storage period.

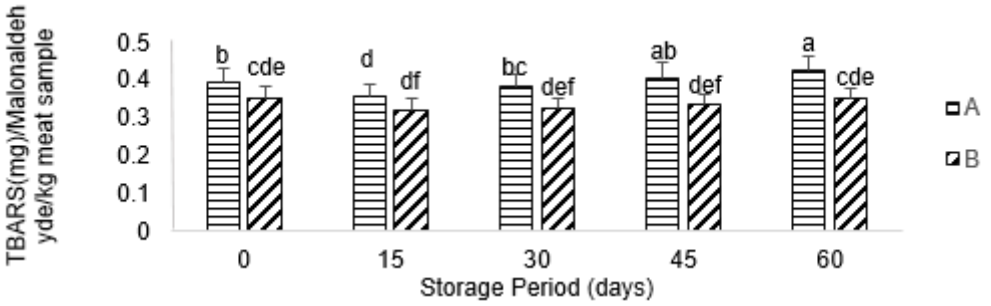

Figure 1. Lipid oxidation of cooked cow blood sausages at -10°C over 60 days storage period.

Treatment A=Sodium chloride, B=Sodium nitrites. Error bars represent standard deviations of means. Means with the same letter are not significantly different at the 5% level. Treatment (P=0.002), Day (P<0.001), Interaction Tmt x Day (P=0.137).

Table 1. Colour changes (mean ± standard deviation) of cooked cow blood sausages stored at -10°C for 60 days.

Cp	Tmt	Storage period (days)					P-values		
		0	15	30	45	60	Tmt	Day	Tmt x Day
L*	A	29.22 ± 3.59	28.59 ± 2.35	30.84 ± 4.71	26.85 ± 2.90	29.84 ± 4.30	0.614	0.168	0.779
	B	30.75 ± 4.05	29.67 ± 2.54	30.35 ± 1.24	28.39 ± 1.66	29.02 ± 3.38			
a*	A	8.15^{c} ± 1.80	8.47^{bc} ± 1.18	8.49^{bc} ± 2.32	9.72^{ab} ± 1.74	8.10^{c} ± 1.80	**0.018**	0.258	0.659
	B	10.37^{ab} ± 2.08	10.67^{a} ± 1.34	10.78^{a} ± 1.06	11.07^{a} ± 1.87	11.08^{a} ± 2.15			
b*	A	7.86^{bc} ± 1.24	7.71^{a} ± 0.71	8.92^{ab} ± 1.90	8.85^{ab} ± 2.05	7.66^{abc} ± 1.50	0.824	**0.031**	0.699
	B	7.67^{bc} ± 1.04	7.48^{a} ± 0.94	8.50^{ab} ± 1.10	8.33^{ab} ± 1.34	8.24^{abc} ± 2.10			

Cp= Colour parameters, Treatment (Tmt) A=Sodium chloride, B=Sodium nitrites.
Values with the same superscript for a colour parameter are not significantly different at the 5% significance level.

IV. CONCLUSION

Incorporating curing salt (Prague powder) in blood sausage formulations improved oxidative stability and enhanced colour retention compared to formulations with common salt.

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DOES A FREEZE-THAW CYCLE AFFECT THE QUALITY OF SOUS-VIDE BEEF?

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I. INTRODUCTION

Freezing is a well-established process widely recognized for extending the shelf-life of meat. Industrially, the utilization of frozen meat as a raw material has become prevalent in recent years due to various advantages such as the acquisition of a sufficient quantity of homogeneous batches, control of market prices, and consistency of the quality attributes [1]. Particularly for sous-vide products, freezing meat in proper portions suitable for sous-vide cooking increases their ease of use (it enables heating only a required number of meat pieces instead of heating more portions than needed). However, freezing might have an adverse effect on beef tenderness, juiciness and meat taste [2], it also increases cooking loss and decreases the lightness and redness of beef [3]. On the other hand, freezing has been observed to result in a decrease in WBSF in cooked meat [2,3]. However, there is a shortage of information about the influence of freezing on the quality of sous-vide beef products. Therefore, the present study aimed to investigate the influence of freezing on the quality of sous-vide beef steaks.

II. MATERIALS AND METHODS

The study was performed on *longissimus lumborum* muscles obtained from 8 Polish Holstein-Friesian bulls (slaughtered at the age of 23 months, 700 kg of live weight). From each muscle, two sub-samples were cut and vacuum-packed individually. All samples were aged to 14d post-mortem at 4°C. Next one sub-sample from each muscle was sous-vide cooked (4h at 60°C) and its quality was assessed. The remaining samples were frozen (-21°C) and kept frozen (-21°C) for 2 months and then thawed (at 7°C for 24h), sous-vide cooked and assessed. The following quality attributes were assessed: pH of raw meat [4], cross-section color of sous-vide cooked beef steaks in the CIELab color space [5], thaw and cooking losses [4], Warner-Bratzler Shear Force (WBSF) [4], Texture Profile Analysis using Instron 5942 universal testing machine (Instron, Norwood, USA) [6], and sensory quality including aroma intensity (1, imperceptible; 10, extremely intense), juiciness (1, extremely dry; 10, extremely juicy), tenderness (1, extremely tough; 10, extremely tender), and meat taste intensity (1, imperceptible; 10, extremely intense) [4]. Data were statistically analyzed in the Statistica 13.3 program (TIBCO Software Inc., Palo Alto, CA, USA) using the variance components module with freezing as a fixed factor (2 levels: unfrozen (control) beef and frozen-thawed beef) and a carcass as a random factor (8 repetitions). When analyzing the results of sensory evaluation also panelist was included in the model as a random factor.

III. RESULTS AND DISCUSSION

The average thaw loss in samples which were frozen after ageing was 6.6%. As a result of freezing the value of pH in raw meat increased from 5.5 to 5.6 ($P < 0.05$). There was no difference in cooking losses between samples processed directly after ageing and samples stored frozen (23.6% vs. 22.8%, respectively). Freezing and thawing affected the color of sous-vided cooked beef, which was

manifested in a significant increase ($P < 0.05$) in lightness, redness, yellowness, chroma and hue angle. Treatments showed similar values of WBSF (30.1 N and 33.0 N, for control and frozen samples). The values obtained indicate that sous-vide cooked beef samples were tender regardless of the storage method before cooking. However, freezing and thawing affected some texture attributes such as hardness 1, and hardness 2, which were higher in frozen-thawed samples, and cohesiveness, which was higher in control samples (Table 1). In contrast, adhesiveness, chewiness and springiness were not affected by freezing. Products obtained from unfrozen beef were scored higher in sensory evaluation in terms of juiciness and tenderness (Table 1), whereas there were no differences between treatments in intensity of meaty aroma and taste. Results obtained highlight the impact of freezing on the quality of meat products, especially on the textural properties and sensory quality. Increased hardness and decreased juiciness were the result of changes in muscle structure. During the freezing of beef ice crystals are created and proteins undergo denaturation, which changes the muscle fibers structure [1,7]. This affects water-holding capacity, decreases moisture in raw muscles and affects the quality of sous-vide cooked meat.

Table 1 – Quality attributes of beef sous-vide products obtained from control (unfrozen) and frozen-thawed *longissimus lumborum* muscle (mean values ± SEM)

Attribute	Control (unfrozen)	Frozen-thawed	P-value
Hardness 1, N	21.1 ± 1.4	32.8 ± 2.2	*
Hardness 2, N	24.4 ± 1.6	38.2 ± 2.6	*
Cohesiveness, -	0.60 ± 0.01	0.54 ± 0.01	**
Juiciness, points	7.02 ± 0.11	5.08 ± 0.15	**
Tenderness, points	7.17 ± 0.16	5.71 ± 0.18	*

* a difference significant at $P < 0.05$; ** a difference significant at $P < 0.01$

IV. CONCLUSION

The freeze-thaw cycle affects the quality of sous-vide cooked beef by changing the color, texture and eating quality of the products. Although, the color changes might be limited by serving products with sauce or after additional thermal treatment, decreased juiciness and increased hardness will reduce consumers' acceptance of the products. Therefore, to obtain good quality sous-vide products it is recommended to use unfrozen beef.

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VOLATILE COMPOUNDS PROFILE OF ONION-GARLIC-BASED FILMS AND COATED BEEF BURGERS

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I. INTRODUCTION

Burgers are usually packaged individually in sealed plastic bags, with a secondary cardboard packaging that combines the burgers into one unit for sale. Films composed of onion and garlic pulp may be appropriate to replace individual plastic packaging bags (primary packaging) and can be prepared together with the burger instead of being discarded; the heating process would solubilize the films, which would be incorporated into the burger, acting as a condiment, which would be another anticipated application [1]. The head-space solid phase microextraction (HS-SPME) technique and identification by gas chromatography and mass spectrometry (GC-MS) of volatile compounds are recommended for verifying the influence on the flavor formation of coated burgers. This study aimed to evaluate the profile of volatile compounds in onion and garlic films and the film's impact on the formation of flavor compounds in coated burgers.

II. MATERIALS AND METHODS

In this experiment, film samples produced with onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) pulp were used in a 4:1 ratio. Commercial beef burgers without salt or spices were purchased. The burgers were wrapped in edible film and kept frozen for seven days, three samples were used.The samples were cooked in an electric grill until an internal temperature of 71°C, then after cooling, they were ground in a food processor. In a glass flask with a capacity of 60 mL, 1 g of the ground sample was weighed and the extraction of volatile compounds was performed by the technique of microextraction in solid phase (SPME) using a carboxen/polydimethylsiloxane (CAR/PDMS) fiber as stationary phase. Gas chromatography coupled to mass spectrometry (GC-MS) was used to separate and identify volatile compounds in the samples, using a DB-5 MS column (5% phenyl, 95% dimethylpolysiloxane) 60 m x 0.25 mm internal diameter and one µm stationary phase thickness. The oven temperature started at 40 °C, increasing 4 °C min-1 to 180 °C, 10 °C min-1 to 280 °C, remaining at this temperature for 5.3 min. Helium (He) was used as carrier gas. The compounds were identified through their spectra and compared with those of the NIST library database. For volatile compounds identification confirmation, an n-alkane (C7-C30) solution (Supelco, Bellefonte, PA) was injected into the equipment under the same conditions as the samples to obtain the programmed linear retention temperature index (LTPRI) of volatile compounds. Experimental identification was performed by comparing the LTPRI and mass spectra with literature reports, with a minimum similarity of 85%. A qualitative analysis was applied to analyze the obtained data.

III. RESULTS AND DISCUSSION

Fifty-nine volatile compounds were identified in the films, and 96 compounds in the coated beef burger samples from different chemical classes, mainly aldehydes, ketones, esters and sulfur

compounds (Figure 1). Volatile compounds that contain sulfur, such as sulfides and disulfides, contribute to the flavor and pungency of garlic and onions [2] [3]. Seven compounds in common were found between the film and burger samples: allyl mercaptan, a major metabolite of garlic compounds; allyl methyl sulfide (odour garlic); 4-dimethylthiophene; dimethyl disulfide and dimethyl trisulfide are compounds reported in analyses of volatile compounds in meat [4] and are associated with meat flavor and sulfurous odor; allyl sulfide and diallyl disulphide have an onion and garlic odor.

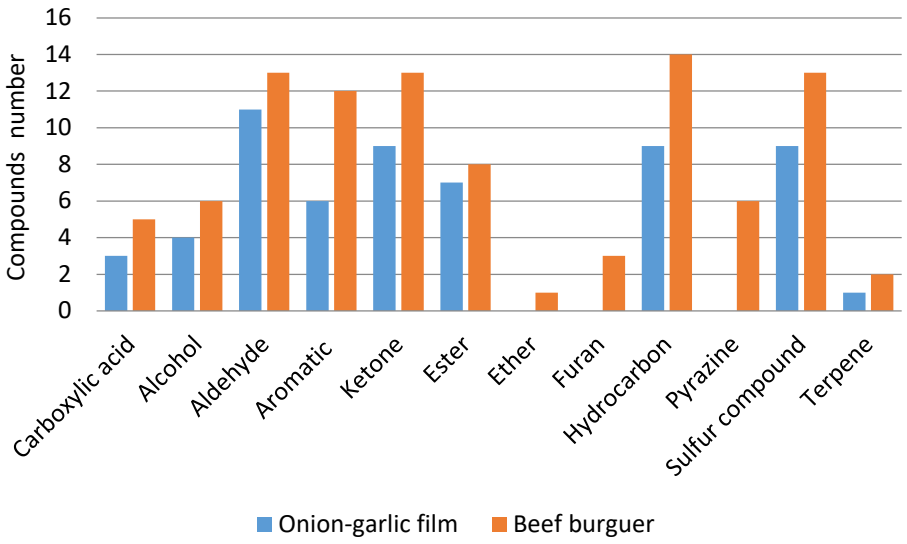

Figure 1. Chemical class found in onion/garlic films and coated beef burgers

IV. CONCLUSION

Applying onion and garlic-based films to beef burgers influenced the formation of aroma in the product, increasing the amount of sulfur-containing compounds which are found mainly in garlic and onion.

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Effect of high pressure processing on the physicochemical properties and sensory evaluation of low-salt pork gels.

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I. INTRODUCTION

Excessive intake of sodium chloride (NaCl) is known to elevate blood pressure, a significant risk factor for cardiovascular and kidney diseases. Between 1982 and 2015, there were 10,414 participants (ranging in age from 18 to 88 years, of whom 50% were female) from 34 different countries, with twelve studies from European and North American countries; twelve from Asian countries; and one from each of the following countries/regions: Australia, New Zealand, Brazil, and the Seychelles Islands; as well as one region with a mixed population. The average salt intake per person was 9.3 grams per day, based on 24-hour urine samples [1]. The World Health Organization (WHO) recommends a limit of 5 g/day of NaCl. Approximately 77% of consumed sodium originates from processed foods [2], with meat and meat products contributing 20-30% of common salt intake [3,4]. Sodium polyphosphate, containing 31.2% sodium compared to 39.3% in NaCl, is typically used at 0.5% compared to salt's 2-6% usage rate in meat products [4,5].

Meat products represent a significant portion of high-pressure processing (HPP) in the food industry, comprising 25-30% of all high-pressure processed foods [6]. High hydrostatic pressure treatment offers opportunities for enhancing the functional properties of myofibrillar proteins and reducing salt and/or phosphate content in gel-type meat products. Studies have shown that HPP has a similar effect on myofibrillar proteins as salt or phosphate, reducing NaCl and sodium phosphate (SPP) [7]. For instance, pressurization at 200 MPa before heat treatment boosted the rheological properties of low-salt (1%) pork sausages [8]. Similarly, HPP following cooking produced low-salt (1%) beef sausage with improved texture and sensory acceptability [9]. Controlling the pressure gradient significantly impacted the functional properties of pork meat batters, enabling a 50% reduction in salt content without compromising product quality [10]. Apart from these studies, in addition to salt pressurized gel-type pork has not been investigated.

Therefore, the objective of this study was to investigate the effects of HHP treatment combined with sodium chloride and sodium phosphate on the physicochemical properties and sensory evaluation of low salt and low phosphate pork gels.

II. MATERIALS AND METHODS

Canadian frozen pork (pork leg) was utilized in this study. The pork was minced through a 16.0 mm, then 3.2 mm plate using a mincer machine. The minced meat was mixed with varying concentrations of sodium chloride (0-2%) and/or sodium phosphate ($Na_4P_2O_7 \cdot 10H_2O$, 0-0.5%) for each sample. High hydrostatic pressure treatment (0.1-200 MPa, 10 min, at room temperature) was applied, with unpressurized pork gels treated under 0.1 MPa. Following HPP treatment, the pork gels were cooked in a water bath at 80 °C for 30 minutes, and then cooled with ice-cold water until the core temperature reached 20 °C before characterization. Measurement items included texture analyses, rheological characteristics, sensory evaluation, Scanning electron microscopy (SEM), SDS-PAGE analysis and Differential scanning calorimetry (DSC) parameters of meat samples.

III. RESULTS AND DISCUSSION

1. Texture analyses: The highest values of hardness, cohesiveness, breaking stress, and modulus of elasticity were observed under high-pressure treatment at 150-200 MPa. These results also indicate

that the use of high pressure of 150-200 MPa can result in sufficient elasticity of pork gels at low salt concentrations.
2. Rheological characteristics: After high-pressure treatment at 150 MPa, the G' and G" of pork gel containing 1% NaCl and 0.5% SPP were higher than those of pork gel containing 2% NaCl and 0.5% SPP without HPP. This suggests that halving the salt (1% NaCl and 0.5% SPP) at 150 MPa also resulted in stronger gels.
3. SEM: Good network structure formation was observed with 2% NaCl and 0.5% SPP, as well as at 150-200 MPa pressure with half as much salt (1% NaCl and 0.5% SPP), indicating successful network structure formation despite reduced salt concentration.
4. Sensory evaluation: The sensory quality of pork gels was assessed and based on several sensory attributes including eating texture and flavor. The results indicated that when the addition of sodium chloride was reduced by 1%, the 150-200 MPa-pressurized pork gel received the high evaluation for the "elasticity" and " Pleasant taste" items compared to the unpressurized pork gel with 2% salt content. Panelists noted that the pork gel with 2% salt content was very salty although comparable to the taste of commercially available meat products. On the other hand, the pork gel with 1% salt content after high-pressure treatment not only improved the elasticity but also effectively controlled the salt content.
5. SDS-PAGE analysis: Following HPP at 100-200 MPa, a decrease in the density of α-actinin band was observed. The HPP at 150-200 MPa before heating improved the rheological properties of pork sausages by disrupting the structure of muscle fibers and dissociating fibrils.
6. DSC: A gradual decrease in total enthalpy of thermal denaturation of pork with increasing pressure was observed, suggesting easier denaturation of pork myofibrillar proteins.

IV. CONCLUSION

HPP at 150 MPa prior to heating enhanced the texture and rheological properties of pork gels. This study demonstrates that high-pressure treatment around 150 MPa in ready-to-eat meat products may modify food properties, with potential applications extending to similar meat products.

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Evaluation of addition of “ora-pro-nóbis” (*Pereskia aculeata* Miller) as antioxidant in sausages containing mechanically deboned poultry meat during refrigerated storage

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I. INTRODUCTION

The ora-pro-nóbis (*Pereskia aculeata* Miller) is a type of Non-conventional Food Plant (PANC) found in the Brazilian Atlantic Forest, though it is rarely used commercially. Due to its high nutritional value, lack of toxicity, and emulsifying, thickening, and gelling properties, this plant make it attractive for applications in the food, cosmetic, and pharmaceutical industries [1; 2]. However, there is a lack of studies that report the potential antioxidant of this species in meat products. Thus, the objective of this work was to evaluate the antioxidant capacity and color properties of sausages containing mechanically deboned poultry meat (MDPM) with natural extracts during refrigerated storage.

II. MATERIALS AND METHODS

The leaves of ora-pro-nóbis (OPN) plant were freeze-dried (Modulyo, Markham, ON, Canada) at −50°C for 96 h. Rosemary (ROS®) and green tea (GT®) commercial extracts were donated by International Flavors and Fragrances (IFF®). Five treatments of sausages containing MDPM were prepared: CON – control (without antioxidant); ERY (Sodium erythorbate) – 0.05%; OPN – 0,05%; ROS® – 0,05% and GT® - 0,05%. The sausages were stored under refrigeration at 4°C and analyzed at two-time points: 1 and 30 days. Color analysis was conducted using a Colorflex 45/0 spectrophotometer (HunterLab, Reston, VA, USA). Color parameters were expressed as lightness (L*), red color intensity (a*), and yellow color intensity (b*). The lipid oxidation (TBARS) was determined according to Vyncke [3] and expressed in MDA/kg sample. For the statistical analysis of the results, analysis of variance (ANOVA) was performed using the General Linear Model (GLM). The production time and the treatment were considered fixed effects, while the manufacturing repetition was a random effect. The software used was Statistica version 7 (StatSoft, Inc., 2004).

III. RESULTS AND DISCUSSION

The TBARS values of all treatment are shown in Table 1. On the first day, no significant difference was observed among the treatments. However, on 30th day, the addition of ERY and OPN were significantly lower ($p < 0.05$) to TBARS values in the sausages when compared to CON, showing that lyophilized OPN leaves have a significant antioxidant effect in sausages at the studied concentration. ROS and GT were like CON and OPN. This antioxidant effect of lyophilized OPN leaves could be due to the phenolic compounds found [4].

Regarding the color parameters (Table 2), the addition of natural extracts resulted in lower a* values ($p < 0.05$), likely due to the inherent greenish hue of OPN leaves and the brown tones of ROS and GT extracts, and the absence of sodium erythorbate (that serves as a cure accelerator and stimulant to color development) and absence of colorant in the formulations. These results agree with those reported by Lise et al. [5], that also observed a decrease in the same parameters with the addition of OPN mucilage in *mortadella*-type meat product.

Table 1. Effect of extracts and ERY on TBARS (mg MDA/kg) in sausage during refrigerated storage.

Treatment	Refrigerated storage (days)	
	1	30
CON	0.188 ^{ns}	0.303 ^{a}
ERY	0.201 ^{ns}	0.220 ^{b}
OPN	0.147 ^{ns}	0.218 ^{b}
ROS	0.169 ^{ns}	0.245 ^{ab}
GT	0.154 ^{ns}	0.255 ^{ab}
SEM	0.007	0.008
p-value	0.111	0.003

a–b Mean values in the same column with different letters indicate significant difference ($p < 0.05$) while ^{ns} mean within the same column aren't significantly different ($p > 0.05$). SEM: standard error of the mean. *p - value*: significance $p < 0.05$. Treatments: CON: control (without antioxidant); ERY: sausage with erythorbate at 0.05%; T1: sausage with OPN at 0.05%; T2: sausage with ROS at 0.05%; T3: sausage with GT at 0.05%.

Table 2. Color parameters of sausage during refrigerated storage.

Parameters	Days	Treatment					*SEM*	*p-value*
		CON	ERY	OPN	ROS	GT		
L*	1	61.15 ^{b}	62.08 ^{a}	59.22 ^{c}	59.35 ^{c}	61.31 ^{ab}	0.229	0.000
	30	58.90 ^{ns}	59.41 ^{ns}	58.70 ^{ns}	59.20 ^{ns}	59.68 ^{ns}	0.199	0.566
a*	1	6.17 ^{b}	9.56 ^{a}	5.76 ^{c}	5.80 ^{c}	5.27 ^{d}	0.289	0.000
	30	9.06 ^{b}	9.64 ^{a}	7.83 ^{c}	7.96 ^{c}	7.10 ^{d}	0.176	0.000
b*	1	15.58 ^{a}	12.83 ^{b}	15.61^{a}	15.85 ^{a}	15.84 ^{a}	0.218	0.000
	30	12.85 ^{b}	13.09 ^{b}	14.14^{a}	14.35 ^{a}	14.26 ^{a}	0.130	0.000

a–d Mean values in the same row with different letters indicate significant difference ($p < 0.05$) while ^{ns} mean within the same row aren't significantly different ($p > 0.05$); *SEM*: standard error of the mean. *p - value*: significance $p < 0.05$. Treatments: CON: Control (without antioxidant); ERY: sausage with at 0.05% erythorbate; T1: sausage with OPN at 0.05%; T2: sausage with ROS at 0.05%; T3: sausage with GT at 0.05%.

IV. CONCLUSION

Lyophilized OPN leaves have demonstrated potent antioxidant effects comparable to sodium erythorbate, establishing them as a promising natural food additive for use as a preservative in meat products. Furthermore, exploring their potential applications in developing new products is imperative.

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Grumixama (*Eugenia Brasiliensis Lam.*): a Brazilian native fruit as natural antioxidant on beef patties during the refrigerated storage

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I. INTRODUCTION

Lipid and protein oxidation decrease the shelf life of meat products, along with altering the color, which are characteristics associated with freshness for final consumption (Bellucci et al., 2022). In this sense, the Myrtaceae family stands out for bioactive compounds with a potential application in the food industry (Saber et al., 2023), such as Eugenia Brasiliensis Lam., native to the Atlantic Forest. Popularly known as “grumixama” or “Brazilian cherry”, could be a renewable alternative source of natural food colorings with bioactive properties (Albuquerque et al., 2024). Although many studies suggest its use as a potential antioxidant, no studies have been found regarding its application in food products. The objective of this paper is to evaluate the oxidative and color stability of grumixama pulp (GP) in beef patties during refrigerated storage.

II. MATERIALS AND METHODS

After removing the seed, the fruit was homogenized and filtered. Four formulations of beef patties were prepared: CON – control; ERY – 500 mg.kg-1 of erythorbate; GP3 – 3% of pulp; GP6 –¨6% of pulp. The patties were stored under refrigeration at 4°C and analyzed at three-time points: 0, 3, and 6 days. The lipid oxidation (TBARS) was determined by Bellucci et al. (2022) and expressed as mg of malonaldehyde (MDA)/kg of sample. The instrumental color parameters were measured using a ColorFlex45/0 colorimeter (Hunterlab, Reston, United States) and reported in the CIELAB color space. For statistical analyzis, the experimental design was randomized blocks in a split-plot scheme. Analysis of variance (ANOVA) and Tukey's multiple comparison test were performed for cases in which there was significance in the ANOVA, using R software version 4.1.3.

III. RESULTS AND DISCUSSION

For TBARS values (Table 1), ERY, GP3 and GP6 were statistically equal on the third day. However, the addition of 6% of GP showed the lower value on the sixth day when compared to ERY and CON, but was similar to GP3. This antioxidant effect can be explained by the bioactive compounds present in grumixama as described by Nehring et al. (2023), it is high values of flavonoids, phenolic acids, total monomeric anthocyanins, and total proanthocyanidins.

TBARS (mg of f MDA/g sample)	Refrigerated storage (days)			SEM
	0	3	6	
CON	0.178	0.271^{a}	0.331^{a}	0.045
ERY	0.124	0.190^{ab}	0.203^{ab}	0.042
GP3	0.135	0.133^{b}	0.138^{bc}	0.045
GP6	0.117	0.145^{b}	0.115^{c}	0.052
Sig.	ns	*	*	

a–c Mean values in the same column with different letters indicate significant difference (P < 0.05); SEM: standard error of the mean; Sig.: Significance; * $p < 0.05$. Treatments: CON: patties without antioxidant; ERY: patties with sodium erythorbate at 500 mg kg−1; GP3: patties with GP at 3%; GP6: patties with GP at 6%;

Regarding the color parameters (Table 2), due to the purple color of GP, the a* values were significantly higher as its concentration increased ($p < 0.05$), suggesting an increase in the reddish color in sausages. Similarly, Yıldız-Turp et al. (2010) report a rise in a* values when adding plum puree to raw beef burgers. Furthermore, on the sixth day, the a* values remained higher in the treatments added with pulp in relation to the others, indicating a desired maintenance of the red color throughout the storage period. The L* value decreased between treatments possibly due to the replacement of water by pulp. For the b* value, there was no significant difference between treatments.

Table 2. Color parameters of beef patties during refrigerated storage.

Parameters	Days	Treatment				Sig
		CON	ERY	GP3	GP6	
L*	0	46.1	45.8	45.9	44.7	ns
	3	49.4 ^{a}	47.1 ^{ab}	44.7 ^{b}	44.9 ^{b}	*
	6	47.4	47.1	44.4	44.9	ns
	SEM	0.960	0.433	0.441	0.067	
a*	0	13.3	13.7	13.7	14.1	ns
	3	9.75^{c}	10.4^{c}	11.6^{b}	14.4^{a}	*
	6	6.48^{b}	6.93^{ab}	7.85^{ab}	8.27^{a}	*
	SEM	1.969	1.955	1.711	1.995	
b*	0	14.7	14.7	15	15.01	ns
	3	14.2	14.2	12.7	13.6	ns
	6	14.2	13.9	13.6	13.2	ns
	SEM	0.167	0.233	0.669	0.549	

a–b Mean values in the same row with different letters indicate significant difference (P < 0.05); SEM: standard error of the mean; Sig.: Significance; Ns.: Not significant; * $p < 0.05$. Treatments: CON: patties without antioxidant; ERY: patties with sodium erythorbate at 500 mg kg−1; GP3: patties with GP at 3%; GP6: patties with GP at 6%;

IV. CONCLUSION

Grape pulp (GP) exhibit promising antioxidant effects as a viable alternative to sodium erythorbate. Incorporating GP into beef patties at concentrations of 3% or 6% has been shown to enhance color and inhibit lipid oxidation during refrigerated storage.

ACKNOWLEDGEMENTS

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ANTIOXIDANT EFFECT OF MAQUI (*Aristotelia chilensis)* AND MURTILLA (*Ugni molinae*) LEAVES IN THE ELABORATION OF PROCESSED MEAT PRODUCTS.

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I. INTRODUCTION

The meat industry is currently facing the challenge of creating products with healthier ingredients, without losing their safety and sensory characteristics, and without affecting their shelf life. Plant polyphenols play an important role in this respect, as they can act as antioxidants and antimicrobials in meat matrices (1). In Chile there are several native species of great biotechnological interest due to their high concentrations of polyphenols. Maqui (Aristotelia chilensis) and murtilla (Ugni molinae Turcz.) are two of the Chilean endemic species with the ability to retard lipid oxidation and inhibit the growth of microorganisms. According to several studies, maqui and murtilla leaves show higher concentrations of polyphenols and a higher antioxidant capacity than fruits and leaves (2,3). So far, there is no evidence showing the behavior of these compounds in processed meats and meat products. This study investigated the effectiveness of polyphenols from maqui (Aristotelia chilensis) and murtilla (Ugni molinae Turcz.) leaves in enhancing the quality of processed meat products.

II. MATERIALS AND METHODS

Maqui and murtilla leaves were sampled and processed to obtain a fine powder (particle size: 80 µm). Subsequently, a characterization of the chemical composition of the leaves was carried out, including proximal composition, polyphenolic characterization, antioxidant, and antimicrobial activity. Two prototype meat products (sausages and hamburgers) were developed with three inclusion levels of maqui or murtilla leaf powders (500; 1000 and 1500 mg/kg). Subsequently, a study of the effect of the powders on the physicochemical, microbiological, and organoleptic properties of the prototypes was carried out. Analysis of the obtained data employed ANOVA and post-hoc Turkey tests to identify significant differences between prototypes.

III. RESULTS AND DISCUSSION

The maqui and murtilla leaf extracts have a high content of total polyphenols (136.97 and 791.77 mg AGE/g, respectively) and strongantioxidant capaciy. In general, maqui extracts showed higher antioxidant capacity than murtilla extracts (Figure1, 2). Both extracts showed a high inhibitory power against Gram (+) Staphylococcus aureus and Bacillus cereus microorganisms. On the other hand, they did not show inhibition for Gram (-) Escherichia coli and Pseudomona aeruginosa. On the other hand, the inclusion of maqui and murtilla leaf powders did not alter the proximal composition of any of the prototypes studied (sausages and hamburgers). While a slight color change (redness) was observed due to chlorophyll in the powders, this did not negatively impact overall acceptability based on sensory panel scores. In burgers with maqui powder polys, lipid oxidation had been inhibited by approximately 44.5% compared to the control, showing a higher antioxidant power than the synthetic antioxidant. Similar results were observed in the other prototypes studied. In addition, the sausages formulation with maqui leaf powder, the profile of volatile compounds showed a correlation between the increase in the concentration of the powder and the decrease in the aldehyde content on day 21 of storage, corroborating the antioxidant power of the maqui leaves. Regarding the fatty acid profile,

no significant differences were observed between tratments neither in the profiles, nor nutritional indices studied n6/n3; atherogenic index, thrombogenic index and trans fatty acid content. The sensory attributes of the hamburgers treated with maqui and murtilla leaf powders and the sausages with maqui leaf powders were acceptable. Finally, the antimicrobial efficacy of maqui and murtilla leaves was confirmed in the prototype sausages and hamburgers.

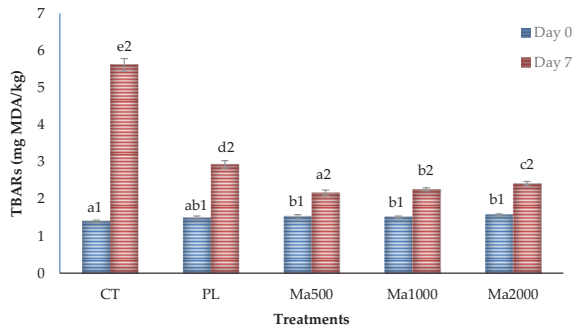

Figure 1. Effect maqui leaf power (MaLP) on lipid oxidation (TBAR value) of beef burgers. CT: no antioxidants; PL: synthetic antioxidant Plus color®; Ma500: 500 mg/kg of MLP; Ma1000: with 1000 mg/kg of MLP; Ma2000: with 2000 mg/kg of MLP.

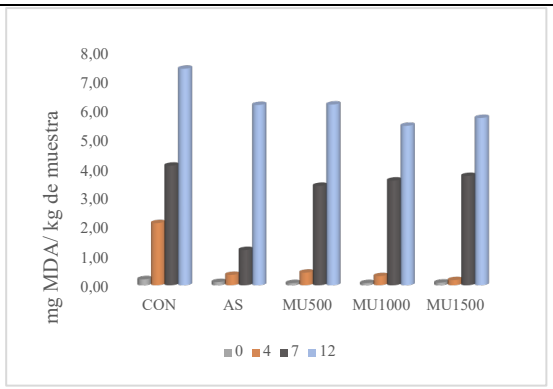

Figure 2. Effect murtilla leaf power (MuLP) on lipid oxidation (TBAR value) of beef burgers. CT: no antioxidants; PL: synthetic antioxidant Plus color®; Ma500: 500 mg/kg of MuLP; Ma1000: with 1000 mg/kg of MuLP; Ma2000: with 2000 mg/kg of MuLP.

IV. CONCLUSION

Maqui and murtilla leaves have high concentrations of natural antioxidants and high antioxidant activity "in vitro" and showed high inhibitory power against Staphylococcus aureus and Bacillus cereus microorganisms. The general acceptability was not affected by the inclusion of maqui and murtilla powders in the prototypes of sausage and hamburgers. The addition of 500 mg/kg of powders of maqui leaves and murtilla to sausage and hamburgers provides a good antioxidant and antimicrobial effect on these fresh meat products.

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PULSED ELECTRIC FIELD (PEF) PRE-TREATMENT TO PROTEIN EXTRACTION FROM PORK LIVER. INFLUENCE ON TECHNOFUNCTIONAL PROPERTIES

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I. INTRODUCTION

Pork liver is considered a high source of protein, around 20%, co-product from the meat industry. Proteins present in this organ have a great nutritional potential due to the composition in essential amino acids [1]. Furthermore, the interesting techno-functional properties of proteins could be suitable as ingredient in the developing of novel foods [2]. Thus, the recovery of pork liver proteins might be an alternative to revalorize the aforementioned co-product. The main procedure to extract proteins consists of alkaline solubilization and further precipitation at the isoelectric point. Pre-treatments using new technologies preceding the protein extraction process could improve protein extraction, as well as to enhance the techno-functional properties of the isolates inducing some modifications in their structure. Thus, the aim of this research was to analyze the feasibility of using pulsed electric field (PEF) pretreatment to improve protein extraction from pork liver and its impact on technological properties of protein isolates.

II. MATERIALS AND METHODS

Pork liver containing 19% of protein was used as raw material. Protein isolates (PI) were extracted from pork liver by pH-shift solubilization pH=8.5) followed by acid precipitation (pH=4) and subsequently PI were freeze-dried. PEF (1 kV/cm, pulse width 25 μs and frequency 10 Hz) was applied as a pre-treatment in the raw pork liver. A Box-Benkhen experimental design (n=3) of 3 factors at 3 different levels was performed by modifying the energy applied in the PEF treatment (0-control, 50, 100 kJ/kg), solubilization temperature (20, 30, 40 °C) and solubilization time (10, 15, 20 min). As response variables, protein extraction yield, protein content and techno-functional properties of proteins (WHC-Water and OHC- Oil Holding Capacity) were analyzed.

III. RESULTS AND DISCUSSION

Protein content in the PI was improved when PEF pre-treatment was applied. Thus, the protein content in control samples (without PEF) was significantly ($p<0.05$) lower (62.0±2.2 g/100 PI) than in PEF treated samples but the effect of the electric energy applied was negligible (73.8±1.9 and 75.8±2.2 g/100 g PI for 50 and 100 kJ/kg, respectively) (Figure 1). Notwithstanding, the PI yield was significantly higher ($p<0.05$) for control samples (53.8±2.0%) than for PEF treated ones (31.4±1.8% and 15.1±2.0% for 50 and 100 kJ/kg, respectively). Using a solubilization pH of 7.5 without pretreatment, Feliu-Alsina [3] found a lower protein content (58.3 g/kg) but a higher PI yield (67 %). Regarding PI technological properties (Figure 1), it was observed that WHC of PI from control samples was lower (2.5±0.1 g water retained/100 g PI) than for PEF ones (2.9±0.1 g water retained/100 g PI for 50 and 100 kJ/kg, respectively). The same trend was found for the OHC, which was higher (3.3±0.3 g oil retained/100 g PI) in PEF pretreated samples when the energy applied was 100 kJ/kg. High values of these parameters are valued to enhance the features of further meat-hybrid products where proteins extracted will be included. Solubilization time and temperature did not affect significantly ($p>0.05$) protein content, protein extraction yield, WHC or OHC.

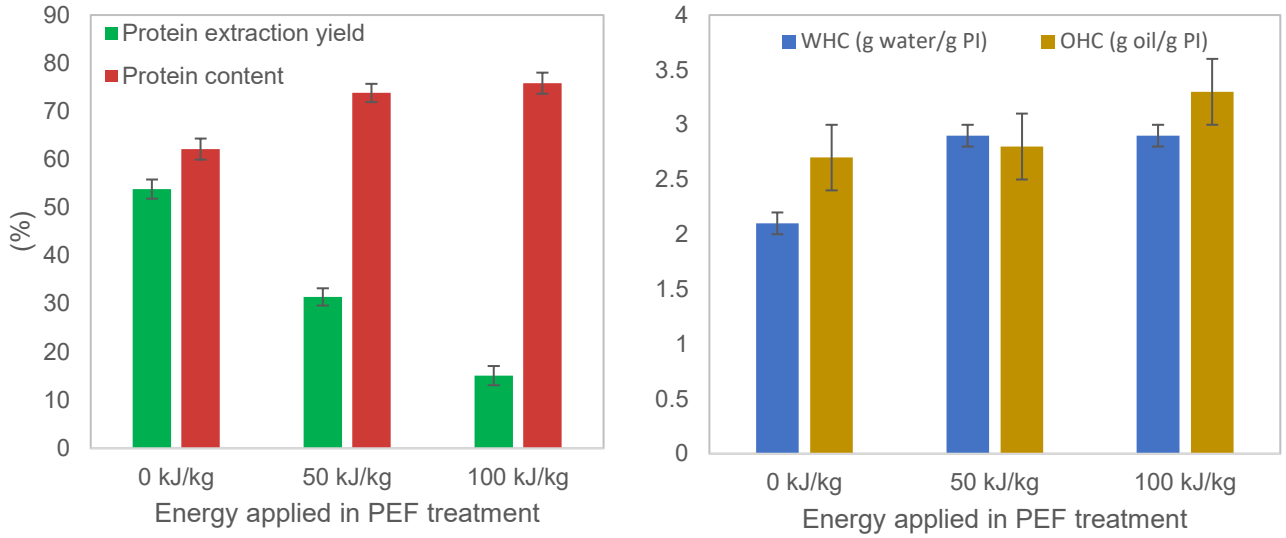

Figure 1 – Protein extraction yield, protein content, and techno-functional properties (WHC and OHC) of proteins extracted from pork liver following a standard pH-shift extraction and PEF pre-extraction (control-0, 50 and 100 kJ/kg).

IV. CONCLUSION

PEF pretreatment during protein extraction improved the protein content of the protein isolate; nevertheless, reduced the protein extraction yield. Furthermore, WHC and OHC were enhanced in the protein isolate obtained with PEF pretreatment. Future studies should elucidate the mechanisms linked to the PEF application on the pork liver which are responsible of the differences observed in the protein isolates analyzed in the present work.

ACKNOWLEDGEMENTS

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PLEUROTUS OSTREATUS CULTIVATED IN AGROINDUSTRIAL WASTES COULD BE USED AS AN ANTIOXIDANT ADDITIVE IN MEAT PRODUCTS

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I. INTRODUCTION

The search for natural alternatives to synthetic antioxidants responds to the growing demand for healthier and more sustainable meat products. Some synthetic antioxidants are associated with adverse effects on human health when consumed in uncontrolled quantities, while some of these can generate toxic byproducts during processing or storage [1]. In this context, *Pleurotus ostreatus* known as oyster mushroom, exhibits valuable antioxidant properties for use as a meat product additive, and these properties are related to their bioactive compounds that can scavenge or reduce free-radical and radical-cations from macromolecules. However, its chemical composition and antioxidant properties may be modified by the type of substrate used for its growth [2]. Thus, the aim of this study was to evaluate the effect of replacing wheat straw with spent coffee grounds (SCG) and potato peels residue (PPR) in the substrate's formulation on the phenolic composition and antioxidant properties of *P. ostreatus*.

II. MATERIALS AND METHODS

P. ostreatus (IE-8 strain) was grown using wheat straw as basal substrate and mixed at different ratios of supplementing residues, as follows: Control, wheat straw at 100%; T1, wheat straw at 80% + 10% of SCG + 10% of PPR; T2, wheat straw at 70% + 15% of SCG + 15% of PPR. Thereafter, fruiting bodies were dried at 60 °C for 12 h and then pulverized at 20 mesh of particle size. Bioactive compounds were extracted from the mushroom powder using water as an extraction solvent. The extracts were subjected to polyphenols content evaluation (phenolics, TPHC; tannins, TTC; chlorogenic acid, CGA). Also, free-radical and radical cation scavenging activity (FRSA and RCSA), as well as reducing power ability (RPA) were tested. Butylhydroxytoluene (BHT) was used as a positive control. Minced pork (*M. semimembranosus*, 24 h *postmortem*; 1.5% salt; 10% fat) was used as meat ingredient in four formulation treatments: Control, without antioxidant; T1 and T2 at 500 ppm, extracts from *P.* ostreatus grown as previously mentioned; BHT, synthetic antioxidant at 500 ppm), cooked in a water bath (65 °C for 60 min), and subjected to pH and thiobarbituric acid reactive substances (TBARS) assays [3,4]. Data (n=6) were subjected to ANOVA and Tukey-Kramer's multiple comparison tests at $P<0.05$ (NCSS v11).

III. RESULTS AND DISCUSSION

As depicted in Table 1, T1 showed the highest TTC and CGA values, while T1 and T2 showed the lowest TPHC values ($P<0.05$). With respect to antioxidant activity, the positive control used showed the highest FRSA, RCSA, and RPA values ($P<0.05$); however, mushroom extracts showed higher antioxidant properties when compared to the no-antioxidant control ($P<0.05$). In agreement with the current results, it has been reported that *P. ostreatus* and *P. pulmonarius* grown in wheat straw show FRSA, RCSA, and RPA activities, which were related to the presence of phenolic compounds [4]. Also,

it has been reported that agro-industrial wastes (coffee pulp, rice straw, corncobs, and their mixtures) increased FRSA and RCSA values of *P. djamor* [3].

Table 1 – Polyphenols content and antioxidant activity of *P. ostreatus* water extract.

	TPHC	TTC	CGA	FRSA	RCSA	RPA
Control	20.50±0.45b	34.33±0.52b	22.01±0.88a	55.20±0.64a	25.77±0.61a	0.74±0.01a
T1	16.00±0.89a	46.00±0.89c	26.35±0.50b	61.00±0.89b	46.00±0.89b	0.81±0.01b
T2	16.08±0.90a	25.33±0.52a	22.67±0.68a	61.13±0.72b	46.23±0.64b	0.79±0.02b
BHT	-	-	-	90.17±0.75c	51.40±0.47c	0.96±0.01c

As noted in Figure 1, meat samples treated with the oyster mushroom (T1 and T2) showed higher pH values than the no-treatment control ($P<0.05$). Also, T1 showed the lowest TBARS values ($P<0.05$). In agreement with our results, it has been reported that *P. ostreatus* and *P. pulmonarius* grown in wheat straw, exert a positive effect against lipid oxidation process as indicated by the lower TBARS values [4]. In addition, the inclusion of *P. ostreatus* extracts to pork meat has increased oxidative stability by reducing pH and TBARS changes during refrigerated storage [5].

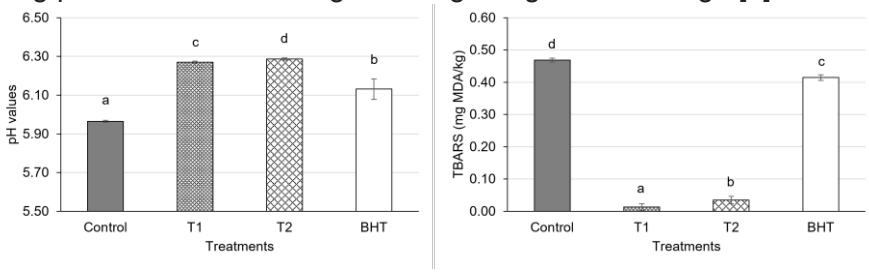

Figure 1. Effect of treatment and cooking period on pH and TBARS values of pork meat.

IV. CONCLUSION

Pleurotus ostreatus is an alternative source of antioxidant compounds that can be used as a natural additive in the meat industry.

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BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF EXTRACTS FROM GREEN COFFEE BEANS OBTAINED WITH DIFFERENT EXTRACTION SOLVENTS AND TIMES

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I. INTRODUCTION

Lipid oxidation is considered one of the main causes of deterioration of meat and meat products, causing a negative effect on sensory quality attributes [1]. One of the objectives of the food industry is to provide consumers with wholesome foods with a minimum of artificial ingredients. Inclusion of natural ingredients displaying molecules with bioactive properties [2] has been one of the current strategies to pursue this pivotal objective. Green coffee beans shown to be an important source of bioactive metabolites such as chlorogenic acid [3]. Thus, the aim of this experiment was to evaluate the phytochemical content and antimicrobial activity of extracts obtained from green coffee beans.

II. MATERIALS AND METHODS

Green coffee beans and textured soy (used as a control) were ground at 80 mesh particle size. The extracts were obtained using different solvents (water, ethanol, and 1:1 water-ethanol mixture) with the ultrasound-assisted extraction method (42 kHz; 25°C), to different extraction times (30 and 60 min). The resulting extracts were evaluated for contents of carbohydrates; total phenols; total flavonoids; caffeoylquinic acid; and condensed tannins. The antioxidant activity of extracts was assessed by inhibition of radical cations through the ABTS; inhibition of free radicals through the DPPH; reducing power using the Prussian blue and FRAP [4]. Data were subjected to analysis of variance (GLM-ANOVA), and treatment means were compared by the Tukey Kramer test at 5%.

RESULTS AND DISCUSSION

As shown in Table 1 the ethanolic extract showed the highest ($p<0.05$) concentration of condensed tannins and a higher content of phenols similar to that of the water-ethanol 1:1 extract, which in turn, showed the highest ($p<0.05$) content of chlorogenic acid and carbohydrates. However, the highest ($p<0.05$) flavonoid content was found in the aqueous extract (Table 1). In general, all extracts had a good response regarding antioxidant activity (Table 2). The ethanolic extract demonstrated antiradical activity against DPPH, ABTS as well as reducing power and FRAP similar ($p>0.05$) to those of ascorbic acid. Likewise, the water-ethanol 1:1 extract presented an antiradical activity against ABTS and a reducing power similar ($p>0.05$) to ascorbic acid.

Soy flours, which are rich in polyphenols and isoflavonoids have been widely used in the formulation of a variety of foods including processed meats to fortifying their nutritional quality, and for improving texture and techno-functional properties [5]. However, in this study a better antioxidant performance of green coffee extracts was observed compared to soy extracts (Table 2). Previous studies [6,7] demonstrated the antioxidant activity and biological activity of green coffee extracts as a result of the polyphenolic compounds it contains.

Table 1 – Comparison of the phytochemical compounds of green coffe extracts.

Treatment	Condensed tannins (% inhibition)	Phenols (mg galic acid equivalents/g)	Flavonoids (mg quercetin equivalents/g)	Chlorogenic acid (abs 700 nm)	Carbohydrates (mg glucose equivalents/g)
1	152.70 ± 12.75^{b}	101.00 ± 4.83^{b}	11.95 ± 1.30^{a}	82.15 ± 2.33^{b}	13.97 ± 1.44^{cd}
2	191.34 ± 12.78^{a}	125.20 ± 5.70^{a}	4.08 ± 0.57^{b}	44.14 ± 2.54^{c}	14.57 ± 0.75^{c}
3	49.56 ± 2.58^{d}	121.65 ± 3.63^{a}	3.70 ± 0.53^{b}	112.00 ± 1.53^{a}	33.00 ± 1.72^{a}
4	43.95 ± 2.81^{d}	22.87 ± 1.72^{c}	-	3.37 ± 0.50^{d}	3.55 ± 0.64^{e}
5	118.99 ± 8.66^{c}	17.68 ± 0.89^{d}	-	3.34 ± 0.55^{d}	11.63 ± 3.32^{d}
6	-	23.96 ± 1.70^{c}	-	2.91 ± 0.22^{d}	26.87 ± 2.24^{b}

T1: Green coffee (Water); T2: Green coffee (etOH); T3: Green coffee (1:1); T4: Soy (Water); T5: Soy (etOH); T6: Soy (1:1).

Table 2 – Comparison of the antioxidant activity of green coffee extracts.

Treatment	DPPH (% inhibition)	ABTS (% inhibition)	Reducing power (mg quercetin equivalents/g)	FRAP (mg Fe^{2+} equivalents /g)
1	30.99 ± 3.27^{c}	84.71 ± 0.44^{b}	1.10 ± 0.03^{b}	102.73 ± 6.96^{c}
2	93.52 ± 0.97^{a}	89.31 ± 0.56^{a}	1.40 ± 0.02^{a}	177.13 ± 2.06^{a}
3	71.35 ± 2.14^{b}	92.06 ± 0.27^{a}	1.41 ± 0.05^{a}	169.26 ± 5.26^{b}
4	19.02 ± 2.70^{d}	51.07 ± 3.30^{c}	0.14 ± 0.03^{c}	18.99 ± 1.26^{d}
5	3.79 ± 1.42^{e}	47.86 ± 4.74^{c}	0.05 ± 0.02^{d}	13.15 ± 2.78^{e}
6	1.23 ± 0.47^{e}	80.98 ± 5.73^{b}	0.06 ± 0.02^{d}	14.56 ± 1.63^{de}
7	92.06 ± 0.89^{a}	93.08 ± 0.26^{a}	1.40 ± 0.03^{a}	173.45 ± 3.05^{ab}

T1: Green coffee (Water); T2: Green coffee (etOH); T3: Green coffee (1:1); T4: Soy (Water); T5: Soy (etOH); T6: Soy (1:1); T7: Ascorbic acid.

III. CONCLUSION

The results indicate that green coffee extracts can be used as an ingredient of natural origin to complement or strengthen antioxidant properties of textured soy in the formulation of meat products.

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Inhibition of Listeria monocytogenes in RTE Beef Strips using NATPRE T10, Celery Powder and Nitrite

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I. INTRODUCTION

In the last decade, there has been continuous progress in evaluating ingredients for processed meats using natural ingredients designed to satisfy consumer preferences and microbiological standards for natural processed meats (*1*). Curing meat products is important not only for product quality but also for safety. Currently, nitrites are used to cure most meat products as well as celery powder, which also contains nitrites (*2*). A natural bacteriostatic alternative, Prosur NATPRE T-10 is currently applied to achieve similar curing characteristics in processed meats. Comparing the product quality is an important aspect of implementation in the meat industry as a curing alternative (*3*). This study was designed to assess the bacteriostatic capability of a novel, natural ingredient (Prosur NARPRE T10) in RTE meat strips by studying its ability to prevent the outgrowth of *Listeria monocytogenes* without compromising its savory flavor compared to the use of nitrite and celery powder.

II. MATERIALS AND METHODS

Ready to Eat meat strips with different ingredient concentrations: 0.52% celery, 0.25% nitrites, or 1% Prosur NATPRE T10, and a negative control/uncured, were produced. Three replicates of strips (5 samples/time point/treatment/replicate) were inoculated with 250µl of a multi-strain mixed Listeria monocytogenes cocktail to yield a 2-3 log concentration on a 25g meat strip. After inoculation, each strip was vacuum-sealed individually in polybags and allowed to attach for 20 minutes. Packaged samples were incubated at a time temperature abuse of 32 °C. Samples were removed at 0, 6, 12, 24, 48, 72, and 96 hours. Samples were hydrated with 225ml of BPW, homogenized by hand for 30 seconds, and serially diluted. then plated in duplicate on an overlay of tryptic soy agar (TSA) on Oxford agar base modified (MOX) supplemented with Moxalactam (Remel™) (*4*). All means were compared using a one-way analysis of variance (ANOVA) with a 95% confidence level and a Tukey's multiple comparison test for significantly different means ($p < 0.05$) in R software 4.2.3.

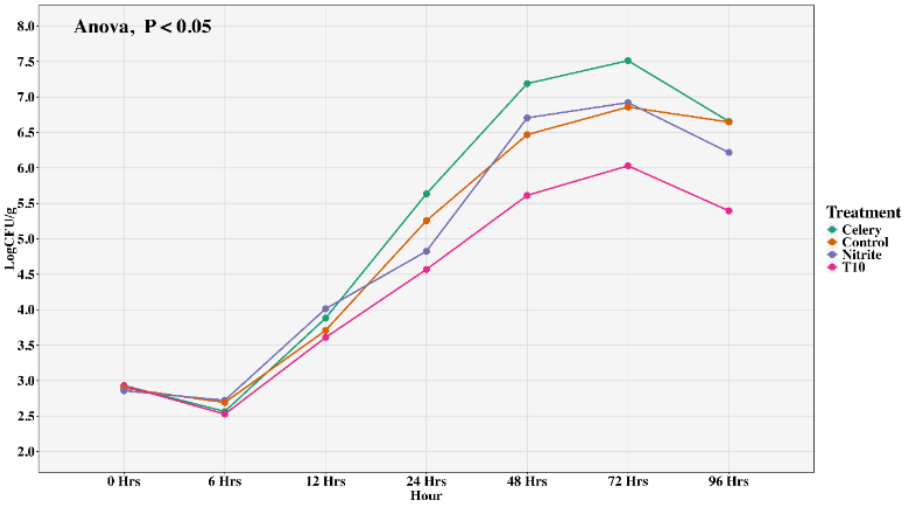
III. RESULTS AND DISCUSSION

L. monocytogenes counts in smoked beef strips are illustrated in Table 1 and represented in Figure 1. Plate counts for all treatments at 6 and 12 hours were not statistically different ($p > 0.05$) in growth compared to time 0 within each treatment over time. However, meat strips prepared with Prosur NATPRE T-10 showed statistically lower ($P<0.05$) LogCFU/g counts at 24, 48, 72, and 96 hours compared to the other formulations. Counts were statistically lower ($p < 0.05$) on average by 1.06, 1.58, 1.48, and 1.26 LogCFU/g at 24, 48, 72 and 96 hours, respectively. Similar results were observed in a cooked ham study cured with natural ingredients (*5*).

Table 1. *L. monocytogenes* growth on beef strips produced using NATPRE T10, nitrites, celery powder, and negative control over time during storage at 32°C.

	***L. monocytogenes* plate counts (Log CFU/g) (Mean ± SE*)**				***P*-value**
Time	**NATPRE T10**	**Nitrites**	**Celery**	**Control**	
0h	2.93 ± 0.10^{a}	2.86 ± 0.08^{a}	2.93 ± 0.09^{a}	2.89 ± 0.09^{a}	0.939
6h	2.53 ± 0.11^{a}	2.72 ± 0.10^{a}	2.56 ± 0.10^{a}	2.69 ± 0.10^{a}	0.496
12h	3.61 ± 0.17^{a}	4.01 ± 0.15^{a}	3.88 ± 0.10^{a}	3.71 ± 0.23^{a}	0.363
24h	4.57 ± 0.29^{b}	4.82 ± 0.16^{ab}	5.63 ± 0.20^{a}	5.26 ± 0.19^{ab}	0.006
48h	5.61 ± 0.14^{c}	6.71 ± 0.22^{ab}	7.19 ± 0.15^{a}	6.47 ± 0.16^{b}	< 0.001
72h	6.03 ± 0.13^{b}	6.92 ± 0.18^{a}	7.51 ± 0.19^{a}	6.86 ± 0.21^{a}	< 0.001
96h	5.4 ± 0.17^{b}	6.22 ± 0.32^{ab}	6.66 ± 0.34^{a}	6.65 ± 0.35^{a}	0.017

Figure 1. *L. monocytogenes* counts (LogCFU/g) on four beef strip treatments at different time points (n= 15 samples/beef strip/sampling point).



IV. CONCLUSION

The clean label fruit and spice extract NATPRE T10 demonstrated better control over the outgrowth of *Listeria monocytogenes* compared to traditional synthetic nitrites.

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VALORIZATION OF *PLEUROTUS GENUS POWDERS* AS POTENTIAL ADDITIVES IN MEAT PRODUCTS

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I. INTRODUCTION

The food industry is constantly looking for natural and healthy alternatives to improve the quality and safety of its products. In this context, food additives play a crucial role in offering physicochemical, techno-functional, and functional properties. A food additive is defined as any substance intentionally added to food for the purpose of improving its characteristics during production, processing, storage, or packaging [1]. Pleurotus mushrooms, commonly known as oyster mushroom, are widely recognized for their nutritional and functional properties which can offer a natural and sustainable alternative [2,3]. In this study, the potential of powders derived from the genus Pleurotus as a meat product additive was explored.

II. MATERIALS AND METHODS

Two Pleurotus strains (*P. citrinopileautos* and *P. djamor*) were grown using wheat straw as substrate (photoperiod 12 h light-12 darkness/28 °C/80-90% relative humidity/<1,200 ppm CO_2) until fruiting bodies were obtained. Both mushrooms were dried at 60 °C for 12 h and then pulverized at 20 mesh of particle size. Pleurotus powders and texturized soy (control) were tested for physicochemical (pH and Hex color), and techno-functional properties, including water and oil holding capacities (WHC and OHC), swelling capacity (SWC), and gel-formation capacity (GFC). In addition, total phenolic, total flavonoids, and total tannins contents (TPHC, TFC, and TTC) were determined. Concerning antioxidant activity, free-radical scavenging activity (FRSA), ferric-reducing antioxidant power (FRAP), and thiobarbituric-acid reactive substances (TBARS) assays were used [4]. Data (n=6) were subjected to ANOVA and Tukey-Kramer's multiple comparison tests at $P<0.05$ (NCSS v11).

III. RESULTS AND DISCUSSION

Pleurotus powders showed higher ($P<0.05$) pH values than texturized soy (Table 1), and Hex color codes indicate the color for all treatments was tan. With respect to techno-functional properties, the highest WHC and GFC values were respectively reached by *P. djamor* and texturized soy. *P. djamor* showed the highest OHC values while the highest SWC values were shown by texturized soy ($P<0.05$).

As depicted in Table 2, no significant differences were found in TTC and TPHC between Pleurotus powders, while TFC was not detected (N.D.) ($P>0.05$). Also, the highest FRSA and FRAP values were displayed by BHT (control) ($P<0.05$). In addition, the lowest lipid oxidation levels of pork meat subjected to 65 °C for 1 h were observed in samples treated with the Pleurotus powder ($P<0.05$).

Table 1 – Physicochemical and techno-functional characterization of edible mushroom powders.

Item	pH	HEX Color	WHC	OHC	SWC	GFC
P. citrinopileatus	7.09±0.01b	#c3a278	49.00±0.89a	38.33±0.52a	24.00±0.89a	27.83±0.68a
P. djamor	7.09±0.02b	#d5b692	71.27±1.27b	54.67±0.52c	37.50±0.45b	57.38±0.48b
Texturized soy	6.49±0.02a	#d7c4a1	70.17±1.13b	48.00±0.89b	84.83±0.68v	57.17±0.26b

Table 2 – Polyphenol's content and antioxidant activity of edible mushroom powders.

Item	TTC	TPHC	TFC	FRSA	FRAP	TBARS
P. citrinopileatus	18.83±1.33a	15.25±0.61a	N.D.	28.83±1.13a	0.28±0.01a	0.35±0.01a
P. djamor	18.33±0.82a	15.33±0.52a	N.D.	28.87±1.54a	0.28±0.02a	0.35±0.02a
BHT	-	-	-	79.96±0.68b	2.00±0.09b	0.39±0.01b

In agreement with our results, it has been reported that powders from *P. ostreatus* and *P. pulmonarious* exhibit significantly higher pH values than texturized soy, with a color described as tan. Both mushroom powders featured the highest OHC values, with no differences in SWC whereas texturized soy showed the highest GFC [4]. Also, phenolic compounds have been found in mushroom powders, which are associated with their antioxidant activity [4,5]. Concerning lipid oxidation, it has been demonstrated that incorporation of mushroom powders to beef patties reduces the TBARS values [5].

IV. CONCLUSION

Pleurotus powders show pH values near neutrality and a tan color. The evidence shows that techno-functional properties of these mushroom powders include WHC, OHC, SWC, and GFC. The presence of phenolic compounds was demonstrated in the samples, as well as their antioxidant activity. In addition, Pleurotus powders showed the highest effect against lipid oxidation of pork meat. Based on the above, edible mushroom powders can be proposed as natural additives for meat products.

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Physicochemical stability of refrigerated cooked cured smoked "Calabresa" sausage

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I. INTRODUCTION

Cooked sausages are meat products manufactured with butcher's animal meat, fat, water, and food additives. They are stuffed in natural or artificial casings and subjected to cooking. "Calabresa" sausage is a very popular product in Brazil. This type of cured sausage uses coarsely ground pork meat and pork backfat as its raw material, is stuffed in natural or artificial casings, and is subjected to cooking in dry and moisture cycles, with smoking optional. The name of the product comes from its characteristic spicy flavor due to red pepper, called Calabresa pepper in Portuguese [1]. In Brazil, sausage is one of the most consumed meat products.

Depending on the microbiological and chemical quality of the raw material, the ingredients and additives added, and the thermal processing cooking cycle, the cooked "Calabresa" sausage may have a short shelf life, mainly due to changes caused by oxidation. It is known that physicochemical stability affects the shelf life of a product. In this sense, the purpose of this study was to evaluate the physicochemical stability of cooked sausage during 60 days of refrigerated storage.

II. MATERIALS AND METHODS

The samples from the different processing stages of the "Calabresa" sausage used for this study (raw meat batter (RB), raw stuffed sausage (RSS), and cooked cured smoked "Calabresa" sausage (CS)) were obtained from a meat industry located in the state of São Paulo (Brazil).

The proximate composition [2, 3], chloride [4], nitrate [5], water activity, and pH value were determined before and after heat treatment of the sausages. The pH was measured using a Digimed pHmeter with a flow-type combined pH electrode, and the water activity (aw) was determined using an AcquaLab® 4TE model (Decagon, Washington, USA). Lipid oxidation was measured spectrophotometrically with thiobarbituric acid reactive substances (TBARS) [6], and instrumental color (L*, a*, b*) was evaluated on days 1 (48 h after processing), 15, 30, 45, and 60 of refrigerated storage (4°C). The residual content of nitrites and nitrates was determined according to Brazil [5].

To evaluate instrumental color, a portable Konica Minolta Chroma Meter colorimeter (model CR 400) was adjusted to the L*, a*, and b* color spaces of the Commission Internationale de l´Eclairage (CIE; "International Commission on Illumination"), with a D65 illuminant (daylight at 6,500 K), a 10° observation angle, and a 30mm shutter opening. Three different points were chosen on the sausage samples (cut in half lengthwise), totaling six measurements. The results were statistically evaluated using ANOVA and Tukey's test with a significance level of 5% using the Statistica® version 10 program (StatSoft Inc., Tulsa, USA).

III. RESULTS AND DISCUSSION

The raw meat batter (RB) presented an aw of 0.971, pH 5.99, and 13.67 and 39.14 mg/kg of nitrate and nitrite content, respectively. Comparing the raw stuffed sausage (RSS) before cooking with the cooked cured smoked "calabresa" sausage (CS), was observed a water loss of around 30%, a decrease in nitrite content of 90% (3.98 mg/kg), a decrease in aw from 0.97 to 0.95, and a pH of around 6.0. The nitrate content in CS remained constant at 17.32 mg/kg. The residual levels of nitrate and nitrite in the samples evaluated demonstrated that the quantities are in accordance with the levels proposed by legislation, which are 300 mg/kg for nitrate and 150 mg/kg for nitrite [1]. A reduction in water activity (aw) can be observed when evaluating raw meat batter, "Calabresa" sausage, before and after cooking and smoking, indicating that the cooking process achieved the desired result in terms of aw reduction in order to guarantee desirable characteristics and a longer useful life, since with a lower aw, deterioration reactions are delayed [7]. The total protein, lipids, ashes, chorides, and moisture content of CS were 19.59; 19.06; 4.64; 2.71; and 54.54%, respectively. According to Brazilian regulations (RTIQ, MAPA), the moisture content of cooked sausages

should be a maximum of 60%. There was no significant difference in the aw of cooked cured smoked “Calabresa” sausage (CS) during 60 days of storage or in the nitrate content (below 22 mg/kg). However, there was a significant difference in residual nitrite (ranging between 2 and 4 mg/kg), but the values are far below what is permitted by legislation (Figure 1). Regarding pH, there was a significant difference ($p < 0.05$), mainly between 1 (6.01) and 60 days (5.84). There was a significant difference in the oxidation index (TBArs); however, the values (0.08–0.29 mg MDA/kg, Figure 2) remained below the threshold of 0.5 mg MDA/kg. In this way, the product did not show considerable rancidity during 60 days of refrigerated storage (Figure 1).

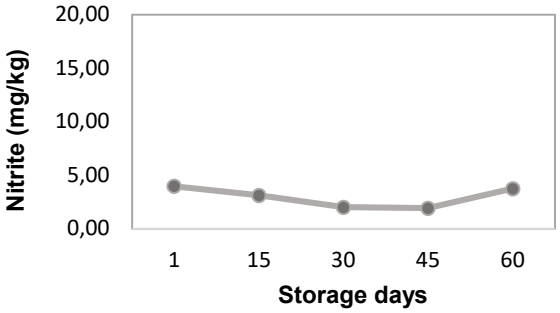

Figure 1. Nitrite content

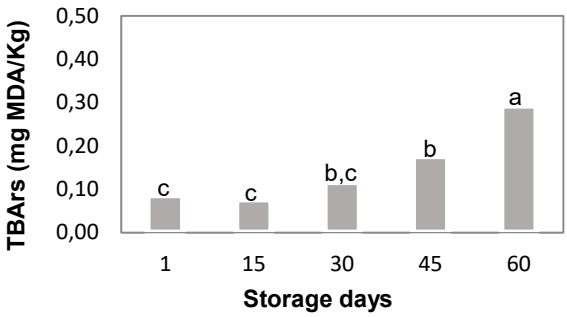

Figure 2. Oxidation index (TBArs)

There were no significant changes in CS color parameters during the 60 days, whose average values were: luminosity (L*) 51.04, red content (a*) 19.91, and yellow content (b*) 8.45. Considering the values of a*, which are higher than the values of b*, which indicate a greater intensity of the red color component and, consequently, indicate a product with a pinkish to redder tone, corresponding to the color normally presented by cooked smoked cured “Calabresa” sausage.

IV. CONCLUSION

In relation to the product evaluated, the cooked cured smoked “Calabresa” sausage's average physical-chemical composition was 19.6% protein, 19.1% fat, 54.5% moisture, and 4.6% ash content. During 60 days of refrigerated storage, this sausage showed no changes in water activity, a slight decline in pH, nitrite content between 1.92 and 9.98 mg/kg, nitrate content from 13.59 to 21.22, and a low index of lipid oxidation (TBArs), which suggests the high stability of this product.

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EFFECT OF NATURAL ANTIOXIDENTS ON THE TEXURAL PROPERTIES OF GAME MEAT PATTIES DURING STORAGE

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I. INTRODUCTION

Game meat production, in South Africa, is faced a significant loss due to waste during harvesting and processing. To reduce waste and improve the utilization of meat, meat restructuring can be applied. During meat restructuring, ingredients such as antioxidants, salt, and soya protein isolates can be incorporated in the meat to improve the meat's flavor and shelf-life (1). The benefit of restructuring meat is that it allows for the use of low-quality meat or trimmings that can be processed into even more valuable products (2) and this helps reduce waste. In this study, the natural antioxidants (black pepper and rosemary) were used in place of Butylated hydroxytoluene in Springbok meat patties and their Textural properties and water activity were analyzed.

II. MATERIALS AND METHODS

Springbok meat trimmings were used to produce patties incorporated with black pepper (15%) and rosemary (15%). Three formulations (each formulation had 0.5% transglutaminase, 2% salt) with treatment one formulation containing a synthetic antioxidant (0.1%) (BHT) was treated as control. The treated meat treatments were stuffed into a plastic casing (diameter × height = 50 mm×70 mm) for the restructuring purpose. The stuffed samples were kept in a refrigerator at 4°C for 4 hours to let the transglutaminase enzyme work. After the 4 hours, the samples were sliced (30 ± 1 g weight, 46 mm × 16 mm) and stored in polyethylene packages (190 × 300 mm) at −40ºC for 15 hours until core temperature reaches to −18ºC. After that samples were stored at −18ºC freezer and 80% relative humidity. About 48 hours later, samples were thawed at 4ºC for 3 hours. The samples were analyzed for textural properties and water activity on day 1, 3, 5 and 7 days. The water activity of the samples was determined using Novasina (Neuheimstrasse, Lachen, Switzerland) water activity meter. The temperature for the chamber system of the meter was set at a temperature of 26ºC. The meat samples that were used had a diameter of 22mm as they were cut by corkborer number 14. An Analysis of Variance (ANOVA) was performed on the data and compared at $P \leq 0.05$ using Fisher's least significant difference (LSD) test following the general linear model (GLM) procedure. Analysis was carried out in (triplicates).

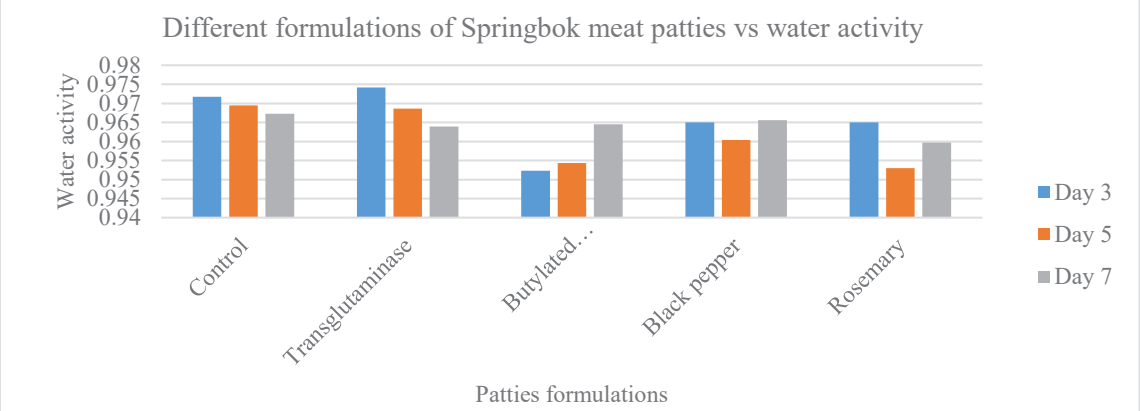
III. RESULTS AND DISCUSSION

Table 1 shows the effects of processing ingredients on the textural petties of Springbok patties. The samples tend to be less springy during storage. There were no significant differences in terms of springiness in relation to the different formulations. This was expected as the same amount of transglutaminase was used in all the formulations. Transglutaminases enzymes catalyze the formation of a peptide bond between lysine or other amino acids and glutamine which may affect protein elasticity. Figure 1 shows the effect of storage on the water activity of the patties. It can be seen that the water activity decreased during storage with the exception of the Butylated hydroxytoluene treated sample which had an opposite effect. This maybe due to the water absorption capacity of the natural antioxidant during storage. This needs further investigation as Butylated hydroxytoluene does not affect water activity because it acts on the lipid phase.

Table 1 – The texture profile of different formulations of Springbok meat patties indicating the springiness and maximum stress.

		Formulations				
		Control	**Transglutaminase**	**Butylated hydroxytoluene**	**Black pepper**	**Rosemary**
Springiness (mm)	**Day 3**	0,96±0,06^{cd}	0,96±0,09^{d}	0,82±0,01^{a}	0,89±0,00^{ab}	0,90±0,03^{bc}
	Day 5	0,43±0,39^{a}	N.D	0,88±0,013^{ab}	0,96±0,03^{bc}	0,97±0,05^{c}
	Day 7	1,04±0,07^{ab}	N.D	1,06±0,048^{bc}	1,14±0,37^{c}	0,99±0,01^{a}

Figure 1. Different formulations of Springbok meat patties vs water activity



IV. CONCLUSION

The study indicates that when natural antioxidants such as black pepper and rosemary are added to restructured Springbok low-value cuts, the resulting meat patties have some benefits to Water activity and textural properties (springiness) when synthetic antioxidants are incorporated during 7-day storage. It is recommended that processors of meat products take into consideration using natural additives like the ones used in this study to improve the physicochemical properties of restructured meat products in accordance with the findings.

ACKNOWLEDGEMENTS
The University of Pretoria is acknowledged for funding the study.

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EXPLORING THE ROLE OF NATURAL ANTIOXIDANTS IN THE DEVELOPMENT OF DIETARY GOAT MEAT PASTES

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I. INTRODUCTION

Kazakhstan dominates the export market for mutton and goat meat, accounting for 91.9% of total exports outside the EAEU. Major markets include the UAE ($3.4 million) and Uzbekistan ($1.6 million). In 2020, significant shipments were also made to Iran ($112,000) [1]. Notably, 70% of goat products are exported as live animals, creating a conflict of interest between farmers and processing enterprises. To address this issue, goat meat paste with antioxidant-rich purslane was developed and investigated.

II. MATERIALS AND METHODS

The object of research was goat meat paste with the addition of purslane and honey. In order to determine the moisture retention capacity, the Grau-Hamm pressing method was used. This method is based on measuring the area of the adsorption moisture spot corresponding to 8.4 mg of water per square centimeter. In order to determine the effect of the natural antioxidant purslane on meat paste, we determined the pH and color stability of the finished product. Determination of the color stability of the finished product. The color characteristics of the samples were determined using a Konica Minolta CM-2300d spectrophotometer calibrated using standard black-and-white calibration plates. The color values were expressed as L-lightness, a-redness and b-yellowness. To determine the color resistance to light, the color stability assessment criterion (Y) was used. Color stability was calculated using the following formula: $Y = \left(1 - \left(\frac{|L_1 - L_2|}{3 \times L_1} + \frac{|a_1 - a_2|}{3 \times a_1} + \frac{|b_1 - b_2|}{3 \times b_1}\right)\right) \times 100,\%$. Where L1, L2 is the value of the light index before and after exposure to light; a1, a2 – the value of the redness index before and after exposure to light; b1, b2 – the value of the yellowness index before and after exposure to light. When determining the color resistance to light, the sample was placed under an artificial light source (an incandescent fluorescent lamp with a power of at least 40 watts). 1 hour after the start of the experiment, the change in color characteristics was instrumentally determined.

III. RESULTS AND DISCUSSION

Our study investigated two types of goat meat paste samples: a control sample without purslane and an experimental sample with purslane powder (1% by weight of minced meat) and honey. The physico-chemical properties of these samples are presented in Table 1.

Table 1 – Physico-chemical analysis of goat meat paste.

Indicators	Control sample	Experimental sample
Mass fraction of moisture,%	61,7	64,8
Mass fraction of fat,%	14,1	10,9
Mass fraction of protein, %	14,8	13,3
Mass fraction of carbohydrates,%	1,2	4,0

The experimental sample with purslane and honey shows a higher moisture content (64.86%) compared to the control (61.7%), attributed to glucose and fructose enhancing moisture retention. The experimental sample also has a lower fat content (10.98% vs. 14.1%) and higher carbohydrate content (4.01% vs. 1.28%) due to the addition of honey and purslane. Moisture binding capacity (MBC) is critical in meat paste production, as it ensures the product's juiciness, tenderness, and pleasant

texture. In this regard, the moisture binding capacity of the control and experimental samples was investigated (Figure 1). Measuring the acidity pH in meat paste helps to control the production processes, ensure the safety and quality of the product, as well as optimize its storage and shelf life (Figure 2).

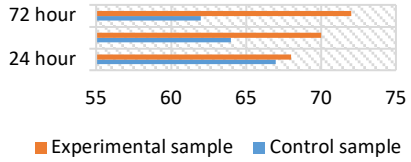

Figure 1. Moisture binding capacity of meat pastes,%

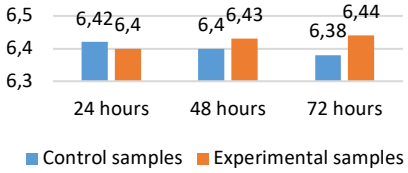

Figure 2. pH acidity of meat pastes

The results (figure 1) show that the addition of vegetable raw materials and honey increases the moisture binding capacity of the finished product. It should also be noted that goat meat has a high potential for moisture binding ability [2]. The results of the pH study (figure 2) showed that the introduction of vegetable raw materials purslane in order to impart antioxidant properties is confirmed by moderate pH values in the experimental sample. When studying the stability before and after exposure to light on the meat paste parameters, the following values were obtained for the main color characteristics: L-lightness, a-redness and b-yellowness.

Table 2 – Color characteristics of goat meat paste.

Samples	Color characteristics before exposure to light			Color characteristics after exposure to light			Color stability, %
	L-lightness	a-redness	b-yellowness	L-lightness	a-redness	b-yellowness	
Experimental sample	70,3±0,3	20,0±0,3	10,9±0,3	69,1±0,3	20,1±0,3	12,5±0,4	93,9±1,7
Control sample	62,9±0,3	17,3±0,4	12,1±0,3	59,6±0,7	17,6±0,4	14,3±0,1	91,7±1,1

The experimental sample with purslane and honey showed superior color stability at 93.9% compared to the control sample's 91.7%. It retained lightness better (98.4% vs. 94.7%) and had a smaller increase in yellowness (12.5 vs. 14.3). Therefore, the experimental sample is the better product due to its enhanced resistance to color changes, maintaining a more appealing and stable appearance.

IV. CONCLUSION

The experimental goat meat paste with purslane and honey demonstrated superior overall quality compared to the control sample. It maintained higher color stability (93.9% vs. 91.7%), better moisture binding capacity at both 24 hours (70% vs. 65%) and 72 hours (72% vs. 60%), and stable pH levels across 24, 48, and 72 hours (6.40, 6.43, and 6.44 respectively) compared to the control sample (6.42, 6.40, and 6.38). The enhanced moisture retention and pH stability indicate that the experimental sample is more effective at maintaining juiciness, texture, and overall product stability. Therefore, the inclusion of purslane and honey significantly improves the quality and shelf life of goat meat paste.

ACKNOWLEDGEMENTS

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MEAT QUALITY AND OXIDATIVE STABILITY OF HIGH-VALUE CHICKEN MEAT CUTS FROM POULTRY FED WITH CANOLA MEAL

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I. INTRODUCTION

Recent research shows very positive aspects of poultry meat as an animal protein of high biological value for human nutrition [1]. The quality of chicken meat, with cuts such as breast, thigh, and leg, which are largely appreciated by consumers, is influenced by diet, management, and processing applied *post mortem*. Corn-soybean-based diets are largely used, with positive effects on the meat quality [2], but the cost negatively impacts the competitiveness of the poultry industry in Uruguay. Canola meal, a co-product resulting from the oil industry of *Brassica juncea or nappus*, with low-glucosinolates, and low-erucic acid, is an alternative source of protein for grower-finisher chickens, when included under 20% [3] with contradictory effects on the meat quality. Consequently, the aim of this research was to study the impact of the inclusion of canola meal, as a partial substitution of soybean meal, in the diet for growing chickens, on meat quality parameters such as p*H,* color, drip loss, and oxidative stability of high-value cuts such as breast, thigh, and leg.

II. MATERIALS AND METHODS

One hundred one-day-old Cobb-500 male chickens were reared until day 20 with a starter diet (22% CP;3000 kcal ME/kg), and at day 21 of age, 96 birds were distributed randomly in four experimental diets (n=24/diet; 21% CP and 3100 kcal ME/kg) *ad libitum,* a corn-soybean basal diet (0% canola meal; CM), and three other corn-soybean diets with 2,5%, 5%, and 10% of canola meal. On day 49, previous fasting, chickens were sacrificed in a commercial slaughterhouse. All procedures followed directives of the Honorary Animal Experimentation Commission, Udelar). After chilling, at 24 hours *post mortem,* p*H*, color, and drip loss were determined in the breast, thigh, and leg [4], then stored in vacuum at -20 °C. p*H* was measured with a Luton pH-201 penetration p*H* meter. For color, the CIE Lab method was applied (CIE,1976; L*; lightness, a*; redness and b*; yellowness) using a Minolta Lab (CR-10) colorimeter with a D65 standard light. Drip loss was determined by weight difference, expressed in %, in samples of each muscle suspended into polyethylene closed bags at +4 °C, after 24 hours. Lipid oxidation was measured through the TBARs described in del Puerto et al. [5]). Briefly, 1.5 grams of frozen meat was homogenized with 20 ml (KCl 0.1M, EDTA 0.02M, BHT0.3mM), with an Ultra-Turrax (IKA T18 Basic) at 8000 rpm for 20 seconds, centrifuged at 2000 *g* for 10 minutes at 4°C.One ml of the supernatant and one ml (0.5 ml, 1% 2-Thiobarbituric acid in HCl 125mM and 0.5 ml TCA 20%) were kept boiling for 30 min, then, placed in ice for 5 minutes and then 45 minutes at room temperature. Afterward, 2 ml of n-butanol was added, vortexed and centrifuged (3000 *g,* 10 min, 4°C). Then, absorbance was measured in a spectrophotometer (T70 UV/Vis, PG Instruments) at 535 nm and concentration of MDA, mg/kg meat, was calculated using its molar extinction coefficient. All data were presented as mean ± SEM. For each variable and cut studied, data was analyzed by ANOVA one way (NCSS software, 2021). When significance was obtained (*p<0.05)*, the Tukey & Kramer test was used. Also an ANOVA GLM was performed with fixed effects of diet and muscle and the interactions.

III. RESULTS AND DISCUSSION

Canola meal inclusion as a partial substitute for soybean meal, at 2.5, 5, and 10% in the grower-finisher diet for chickens did not affect the growth of birds (not included) but modifications in quality parameters were observed in this work. p*H* decreased in breast with 5% of CM and in breast and thigh with 10% of CM (Table 1). Lightness and yellowness decrease in thigh, while in leg only redness decreased related to control (Table 1). The CM inclusion at 5 % and 10%, significantly decreased the drip loss in breast and thigh, whereas in leg all doses of CM caused a decrease in water loss at 24 hours *post*

mortem (Fig 1). The lipid oxidation measured by the TBARS expressed as MDA (mg/kg meat) (Table 2) shows an effect, particularly in leg, with a significant increase in the MDA for the 2.5 % of CM compared to the control. No effect was observed in breast or thigh. Overall effects could be attributed to an increase of lipids content, or an effect of polyphenol content of canola meal. Further research is being done to explain the effects observed in this study.

Table 1. Effect of canola meal (CM; 0, 2.5, 5 and 10 %) in diet on pH and color *L*a*b** in breast, thigh and leg.

CM, %	Breast				Thigh				Leg			
	pH	L*	a*	b*	pH	L*	a*	b*	pH	L*	a*	b*
0	6.09 a	54.7	-0.75	8.09	6.54 a	55.2 a	0.70	9.00 a	6.30	56.4	-0.26 a	7.14
2.5	6.02 a	54.7	-0.94	8.14	6.50 a	53.5 b	1.06	7.37 b	6.31	55.8	-0.28 a	7.61
5	5.98 b	54.2	-1.18	7.88	6.50 a	51.9 c	1.22	6.19 c	6.32	55.7	-0.76 b	7.55
10	5.96 b	55.1	-0.90	8.90	6.43 b	53.6 b	0.70	7.68 b	6.34	56.2	-0.84 b	7.34
p	0.01	ns	ns	ns	0.05	0.01	ns	0.01	ns	ns	0.03	ns

Data are mean of n=24, (SEM not shown). a, b: means significant difference between diets, p<0.05.

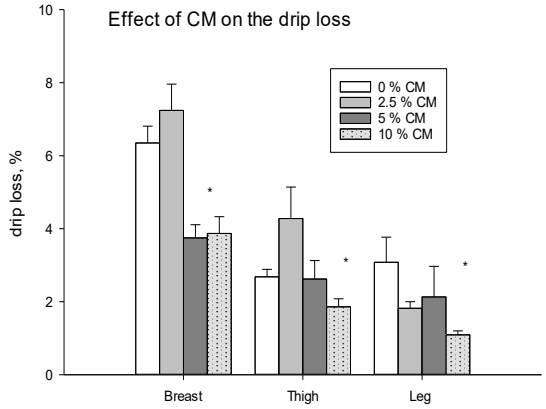

Figure 1. Drip loss (%) in breast, thigh and leg of poultry receiving 0, 2.5,5 and 10 % of canola meal (CM) in diet. Data are mean ± SEM (n=24). *= p<0.05

Main effects:
Diet p<0.01: 0%,2.5%>5%,10%
Muscle: p<0.01:breast>thigh,leg

CM, %	Breast	Thigh	Leg
0	0.30±0.02	0.51±0.05	0.36±0.03 b
2.5	0.39±0.04	0.44±0.04	0.55±0.05 a
5	0.43±0.05	0.53±0.04	0.52±0.05 ab
10	0.38±0.03	0.43±0.04	0.48±0.04 ab
p	ns	ns	0.01

Table 2. Effect of canola meal inclusion (CM; 0%, 2.5%, 5%, and 10%) in diet on lipid oxidation (MDA, mg/kg meat) of breast, thigh and leg. Data are mean ± SEM (n=6). a,b:means significant difference, p<0.05.

Main effects:
Diet p<0.03: 5%CM>0%CM
Muscle p<0.01: breast<thigh,leg

IV. CONCLUSION

Canola meal coming from *Brassica* improved for oil industry included at 2.5 to 10 % in chicken diet, positively impacted the meat quality, reducing the water loss in all studied cuts. The results of the effects on the pH, color, drip loss, and lipid oxidation were largely dependent on the muscle type.

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Potentials of Edible Flowers on Reducing Lipid and Protein Oxidation in Ground Beef

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I. INTRODUCTION

Edible flowers, rich in antioxidants, have been used in traditional medicine and food for centuries and show potential for preserving meat products [1]. Among edible flowers, roses, and hibiscus stand out for their antioxidant properties. Roses, known for their various health benefits, effectively inhibit lipid oxidation in meat products [2]. Similarly, hibiscus extracts, rich in phytochemicals, combat oxidative damage and enhance meat quality and taste. Incorporating these flowers into beef products offers improved quality and nutritional value, making them valuable additions to the industry (Santos et al., 2022). This study evaluates the potential role of rose and hibiscus flowers on lipid and protein oxidation in fresh ground beef stored under simulated retail conditions for seven days. By understanding the mechanisms behind oxidation in beef products and exploring natural antioxidants, this research aims to enhance the quality and shelf life of meat-based products.

II. MATERIALS AND METHODS

The raw ground beef patties were mixed with the treatment ingredients and molded into patties. They were then stored and displayed in a retail display case for 7 days under retail display conditions. This entails dividing the raw ground beef into seven treatments, each consisting of 30 grams: raw ground beef patties (control), raw ground beef patties mixed with 1, 2, and 3 % hibiscus powder, ground beef patties mixed with 1, 2, and 3 % rose powder. All preparation procedures, including mixing, patty formation, packaging, and storage, were conducted at 4°C for intervals of 0, 1, 3, 5, and 7 days. The 1, 2, and 3 percentages focus on parameters such as pH, water holding capacity (WHC), textural attributes, and color. Additionally, the study evaluated oxidative degradation using four oxidation markers (protein carbonyls, Schiff bases, and free thiols), and antioxidant capacity was determined using the DPPH assay.

III. RESULTS AND DISCUSSION

The edible flowers exhibited significant improvements in several key parameters associated with oxidative stability, including increased scavenging of free radicals, enhanced water-holding capacity, reduced TBARS values, decreased protein carbonyls, and improved retention of free thiols and Schiff bases. Additionally, the inclusion of hibiscus and rose powders helped in maintaining favorable color attributes and textural properties in the patties. Our study highlights the beneficial effects of incorporating hibiscus and rose powders at varying concentrations (1%, 2%, and 3%) in raw ground beef patties to mitigate lipid and protein oxidation. These edible flowers exhibited significant improvements in several key parameters associated with oxidative stability, including increased scavenging of free radicals, enhanced water-holding capacity, reduced TBARS values, decreased protein carbonyls, and improved retention of free thiols and Schiff bases. The incorporation of roselle and rose powders led to heightened scavenging activity, resulting in reduced oxidative activity, carbonyls, Schiff bases, and free thiols during storage (Figure 1). Additionally, the inclusion of hibiscus and rose powders helped in maintaining favorable color attributes and textural properties in the patties.

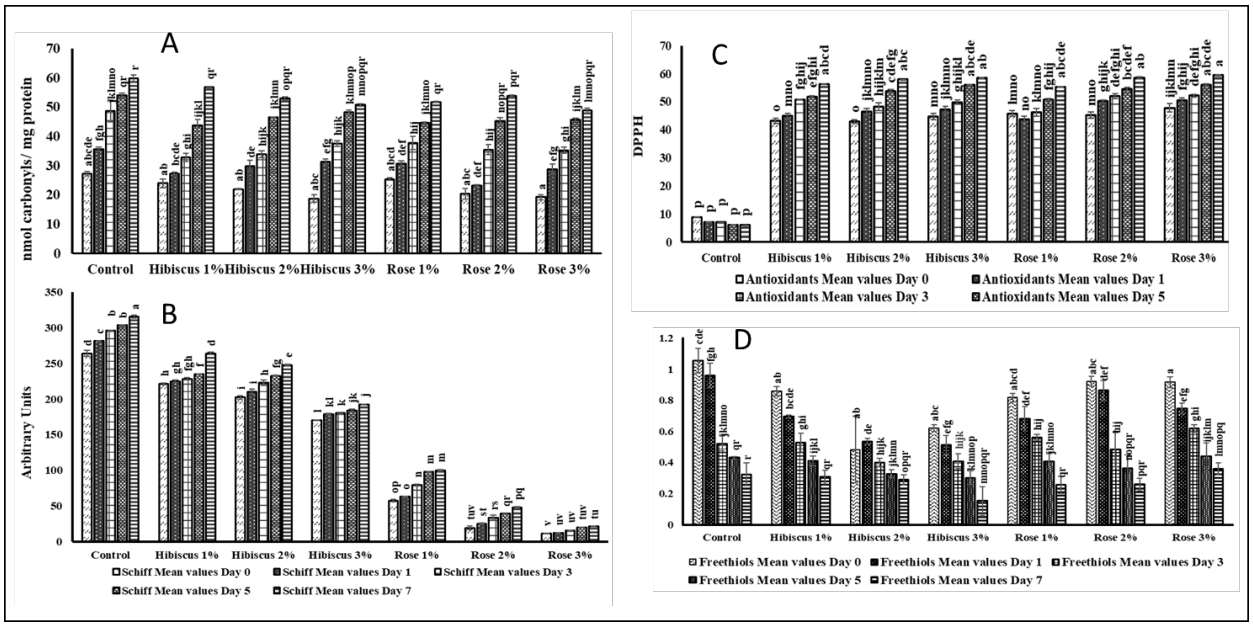

Figure 1. The graph represents the concentration of protein carbonyls (1A), Schiff bases (1B), antioxidant capacity (1C), and free thiols (1D) obtained from Days 0 - 7 with different treatments. Results are presented as mean ± SD of three independent determinations. Letters connected by different letters are significantly different. Significant difference within the treatment ($p < 0.05$).

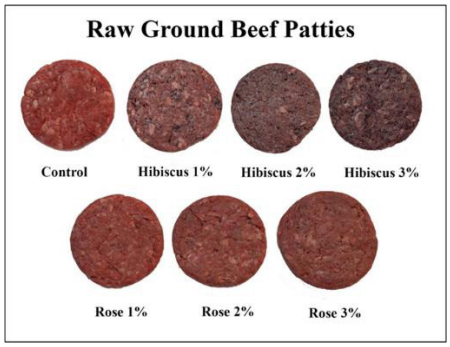

Figure 2. The visual appearance of the raw ground beef patties is evaluated after preparation with seven treatments: Raw ground beef patties (control), ground beef patties with 1, 2, and 3 % Hibiscus sabdariffa L. powder, including ground beef patties treated with 1, 2, and 3 % Rosa canina L. powder.

CONCLUSION

Notably, our findings suggest that higher concentrations of hibiscus and rose powders, particularly at 3% powder, demonstrate the greatest potential in reducing lipid and protein oxidation compared to untreated patties and those treated with lower concentrations. Overall, the inclusion of rose powder exhibited more favorable effects than untreated raw ground beef patties and those treated with roselle. Consequently, raw ground beef patties treated with rose powders demonstrated greater efficacy in enhancing the quality attributes under investigation.

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Application of açaí and celery extracts as antioxidants in Italian-type salami

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I. INTRODUCTION

Consumers increasingly turn food purchasing into a decision-making process, critically evaluating labels, comparing brands, and, most importantly, analyzing ingredient lists. Natural fruit extracts have been widely studied as potential substitutes for synthetic antioxidants in various products. They aim to reduce oxidation reactions that begin at the animal's slaughter stage and influence meat's sensory quality and nutritional value and by-products [1]. This study aimed to evaluate the addition of açaí and celery extracts as natural antioxidants in Italian-type salami, focusing on lipid oxidation during maturation time and post-slicing storage.

II. MATERIALS AND METHODS

To make the açaí extract, water was added to the pulp in a 1:1 ratio, stirred for 30 min at 150 rpm (protected from light), and centrifuged (4500 rpm for 20 min). The supernatant was filtered, frozen, and lyophilized. four formulations of Italian-type salami added with açaí and celery extracts were prepared: **ERI:** formulation with 500 mg/kg of sodium erythorbate, 150 mg/kg of sodium nitrite and 150 mg/kg of sodium nitrate, **EA**: with 150 mg/kg sodium nitrite, 150 mg/kg sodium nitrate and 500mg/kg açaí extract, **EAA150**: with 150ppm celery extract and 500mg/kg açaí extract, **EAA300**: with 300ppm extract of celery and 500mg/kg açaí extract. 0.025mg/kg of Bactoferm® T-SPX starter culture was used in all treatments. The pork meat and pork back fat were ground (8 mm) and mixed with NaCl and other ingredients. The samples of all treatments were stuffed into collagen casings (15 cm in length, 50 mm in diameter). Temperature and relative humidity parameters (T °C and RH%) were controlled following the procedure for 14 days. The lipid oxidation (TBARS) was determined by Vyncke [2] and expressed in the MDA/kg sample.

III. RESULTS AND DISCUSSION

The TBARS values during the maturation time (Table 1) obtained from the treatments with added açaí and celery extracts did not differ from the ERI treatment on days 0 and 14. On day 10, only the EAA300 treatment differed from the ERI treatment, highlighting the antioxidant potential of açaí and celery extracts against lipid oxidation. At the end of the maturation time (days 10 and 14), no significant differences ($P < 0.05$) were observed between them, but the values were significantly higher ($P < 0.05$) at the beginning of processing (day 0).

During storage, the TBARS values (Table 2) obtained from treatments with added açaí and celery extracts did not differ from the ERI treatment on days 0, 7, and 21, except on day 14, when treatments EAA150 and EAA300 presented higher values compared to EA and ERI treatments. All treatments with added natural antioxidants (celery and açaí extracts) did not show significant differences ($P > 0.05$) during the storage period, demonstrating the antioxidant effect of the extracts on the shelf life of Italian-type salami.

Table 1. Lipid oxidation values of Italian-type salami during maturation time.

Treatments	Days				
	0	10	14	SEM	*p-value*
ERI	0,377^{b}	0,619^{Ba}	0,697^{ABa}	0,043	*
EA	0,448^{b}	0,667^{Ba}	0,624^{Bab}	0,035	*
EAA150	0,469^{b}	0,721^{ABa}	0,816^{Aa}	0,044	*
EAA300	0,543^{b}	0,831^{Aa}	0,723^{ABa}	0,036	*
SEM	0,033	0,022	0,023		
p-value	ns	*	*		

$^{A-B}$ Mean values in the same column (different treatment on the same day) with different letters indicate a significant difference (P < 0.05); $^{a-b}$ Mean values in the same line (same treatment on different days) with different letters indicate a significant difference (P < 0.05); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P < 0.05. Treatments: **ERI**: sodium erythorbate with 500 mg kg−1, sodium nitrite with mg kg−1 and sodium nitrate with 150 mg kg−1, **EA**: açaí extract with 500 mg kg−1, sodium nitrite with 150 mg kg−1 and sodium nitrate with 150 mg kg−1, **EAA150**: celery extract with 150 mg kg−1 and açaí extract with 500 mg kg−1, **EAA300**: celery extract with 300 mg kg−1 and extract of açaí with 500 mg kg−1.

Table 2. Lipid oxidation values of Italian-type salami during post-slicing storage.

Treatments	Days					
	0	7	14	21	SEM	*p-value*
ERI	0,718^{a}	0,680^{ab}	0,660^{Bab}	0,594^{b}	0,016	*
EA	0,664	0,673	0,666^{B}	0,652	0,022	ns
EAA150	0,783	0,707	0,722^{AB}	0,689	0,021	ns
EAA300	0,738	0,797	0,796^{A}	0,743	0,024	ns
SEM	0,022	0,024	0,016	0,027		
p-value	ns	ns	*	ns		

$^{A-B}$ Mean values in the same column (different treatment on the same day) with different letters indicate a significant difference (P < 0.05); $^{a-b}$ Mean values in the same line (same treatment on different days) with different letters indicate a significant difference (P < 0.05); SEM: standard error of the mean; Sig.: Significance: n.s.: Not significant; * P < 0.05. Treatments: **ERI**: sodium erythorbate with 500 mg kg−1, sodium nitrite with mg kg−1 and sodium nitrate with 150 mg kg−1, **EA**: açaí extract with 500 mg kg−1, sodium nitrite with 150 mg kg−1 and sodium nitrate with 150 mg kg−1, **EAA150**: celery extract with 150 mg kg−1 and açaí extract with 500 mg kg−1, **EAA300**: celery extract with 300 mg kg−1 and extract of açaí with 500 mg kg−1.

IV. CONCLUSION

The study demonstrates that the incorporation of açaí and celery extracts in Italian-type salami provides an effective antioxidant effect, as evidenced by the TBARS values measured during both maturation and storage periods. The treatments containing these natural extracts showed similar lipid oxidation levels compared to the control treatment (ERI) at most time points. This suggests that açaí and celery extracts are viable natural alternatives for enhancing the oxidative stability of meat products, offering a promising approach for clean label formulations in the food industry.

ACKNOWLEDGEMENTS

The authors would like to thank the National Council for the Improvement of Higher Education (CAPES - Coordenaçao de Aperfeiçoamento de Pessoal de Nível Superior) and CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnologico)for their financial support.

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Açaí processing residue extract as a potential antioxidant in beef patties at frozen storage

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I. INTRODUCTION

Antioxidants are added to processed meats to reduce the oxidative process, and many natural plant extracts can contribute to this [1]. Within this context, açaí (Euterpe oleraceae Mart) has become an alternative source of these antioxidant compounds. Given these considerations, the present study evaluated the antioxidant potential of açaí processing residue extract, applied to beef patties stored under freezing temperature.

II. MATERIALS AND METHODS

The residue was collected, and distilled water was added to obtain the mixture which was homogenized for 10 min., with the use of a propeller stirrer. At that point, water associated with the extracted compounds was obtained. This content was centrifuged, and the supernatant was filtered and freeze-dried. Five treatments were tested: a control with no antioxidant (CON), sodium erythorbate added (ERY), and three more with açaí residue extract (AR): low (L-AR; 750 mg/kg), medium (M-AR;1000 mg/kg) and high (H-AR;1500 mg/kg) concentrations, with three analysis points (30, 60 and 90 days). A ColorFlex45/0 spectrophotometer was used to determine the color parameters. For lipid oxidation, the TBARS index was determined by the reaction between oxidation products and thiobarbituric acid (TBA), forming compounds that can be measured in a spectrophotometer at 532 nm [2].

III. RESULTS AND DISCUSSION

The frozen beef patties presented an increase in malonaldehyde concentration over the storage days (Figure 1). The control treatment (CON) showed the highest TBARS values for all days (0.233, 0.367, 0.413, and 0.568 mg MDA/kg of sample for 0, 30, 60, and 90, respectively). At day 0, CON showed the highest value for mg MDA/kg of the sample, statistically similar to the treatments with low and high concentrations of açaí residue extract (L-AR and H-AR). For the other sampling points (30, 60, and 90 days), ERY and the three different concentrations of açaí residue extract (L-AR, M-AR, and H-AR) were statistically similar, regarding the amount of mg MDA/kg of sample. Regarding the color parameter, the lightness (*L*) was not significantly affected after 30 and 60 days when comparing the five different treatments (Table 1). The lowest values were obtained at 90 days of storage for the M-AR and H-AR treatments (40.1 and 41.5 respectively). The *a** parameter changed only at day 0, with CON treatment obtaining the lowest value (14.8). The other treatments were statistically similar at that same time. The treatments had no significant difference at 30 days for the *b** parameter ($P>0.05$). At 90 days, L-AR and M-AR were statistically similar to ERY. Thus, the three different concentrations of açaí residue extract made a positive contribution to the b* parameter.

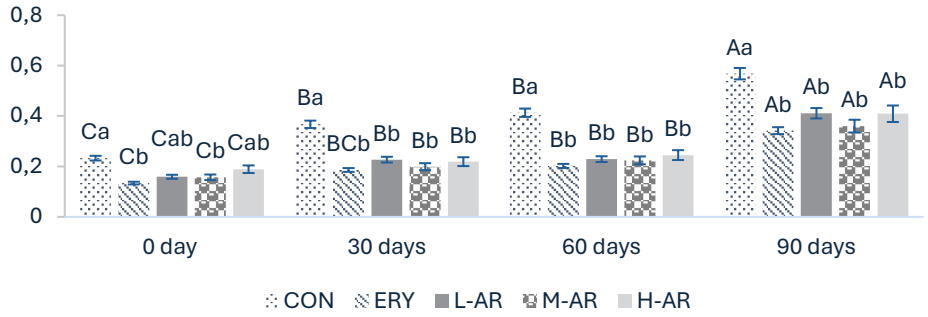

Figure 1. TBARS of beef patties of all treatments.
[a–b] Mean values on the same day for different treatments, with different letters indicating significant difference (P<0.05); [A-B] Mean values for the same treatment on different days with different letters indicating significant difference (P<0.05). Errors bars corresponding to standard error (S.E.).

Table 1. Color parameters of beef patties stored at -18°C.

Parameters	Days	CON	ERY	L-AR	M-AR	H-AR	SEM	Sig
*L**	0	45.5^{Aab}	46.7^{Aab}	45.0^{Ab}	44.1^{Ab}	46.6^{Aa}	0.25	<0.01
	30	44.1^{AB}	45.0^{A}	43.1^{B}	43.0^{A}	42.1^{B}	0.28	n.s.
	60	42.3^{B}	44.1^{AB}	43.0^{B}	43.5^{A}	42.6^{B}	0.33	n.s.
	90	43.0^{ABa}	42.3^{Bab}	43.0^{Ba}	40.1^{Bb}	41.5^{Bb}	0.31	<0.01
	SEM	0.37	0.35	0.21	0.37	0.36		
	Sig	0.04	0.01	0.00	0.00	0.00		
*a**	0	14.8^{b}	16.5^{Aa}	15.3^{ab}	15.4^{ab}	15.3^{ab}	0.22	<0.01
	30	14.4	14.3^{AB}	15.1	14.4	13.7	0.32	n.s.
	60	14.0	12.6^{B}	14.6	14.0	13.8	0.36	n.s.
	90	12.7	12.2^{B}	13.6	12.9	13.5	0.35	n.s.
	SEM	0.36	0.40	0.34	0.39	0.33		
	Sig	n.s.	0.00	n.s.	n.s.	n.s.		
*b**	0	16.2^{A}	16.3^{A}	16.9^{A}	16.5^{A}	16.8	0.10	n.s.
	30	16.0^{A}	16.0^{AB}	16.1^{AB}	16.2^{AB}	16.3	0.11	n.s.
	60	15.4^{Ab}	16.0^{Ba}	15.3^{Bb}	15.5^{ABab}	15.5^{ab}	0.13	<0.01
	90	14.7^{Bb}	14.2^{Cb}	15.9^{Ba}	15.4^{Ba}	14.5^{b}	0.13	<0.01
	SEM	0.14	0.13	0.14	0.15	0.15		
	Sig	0.00	0.00	0.00	0.01	0.00		

a–b Mean values in the same row (for different treatments, on the same day), with different letters indicating significant difference. (P<0.05); A-B Mean values in the same column (on different days, for same treatment) with different letters indicating significant difference. (P<0.05). SEM: Standard error of the mean; Sig: Significance; n.s.: Not significant (P>0.05)..

IV. CONCLUSION

The addition of açaí residue extract in beef patties reduced lipid oxidation, indicating its antioxidant potential in meat products. For the color parameters, the low concentration (750 mg/kg) of açaí residue extract proved to be more effective at the storage temperature.

ACKNOWLEDGEMENTS

The authors would like to thank the National Council for the Improvement of Higher Education (CAPES - Coordenaçao de Aperfeiçoamento de Pessoal de Nível Superior) and CNPQ (Conselho Nacional de Desenvolvimento Científico e Tecnologico) for their financial support.

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ULTRASOUND PRETREATMENT INFLUENCES FLAVOR PROFILE OF DRY-CURED HAM SLICES

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I. INTRODUCTION

Dry-cured ham is produced with the whole hind leg of pig as raw material which is usually sliced, vacuum packaged and stored at 4 ºC for preservation before sale [1]. Nevertheless, the quality (flavor, texture, color, etc.) of dry-cured ham slices is easily prone to be impaired during long term vacuum storage decreasing their acceptability when evaluated by trained panel although not appreciated by consumers [2]. Ultrasound is a promising technology to improve the quality profile of meat products [3]. However, the investigation of ultrasound-induced flavor changes during the storage of dry-cured ham slices is still lacking.

II. MATERIALS AND METHODS

2.1 Sample preparation

The experimental study employed 6 hams from two different dry-cured times (9 m and 12 m), which were submitted to two treatments (control and ultrasound) and vacuum storage at refrigeration for 0, 20, and 40 d. Ultrasound parameters were set as 200 W (water bath, 35 kHz) and 30 min.

2.2 Sensory evaluation and volatile compounds analysis

The determination of volatile compounds in the headspace of dry-cured ham samples was performed by using GC-MS [4].

2.3 Statistical analysis

Data were processed by XLSTAT2018 software, and the significant significance was set as $p < 0.05$.

III. RESULTS AND DISCUSSION

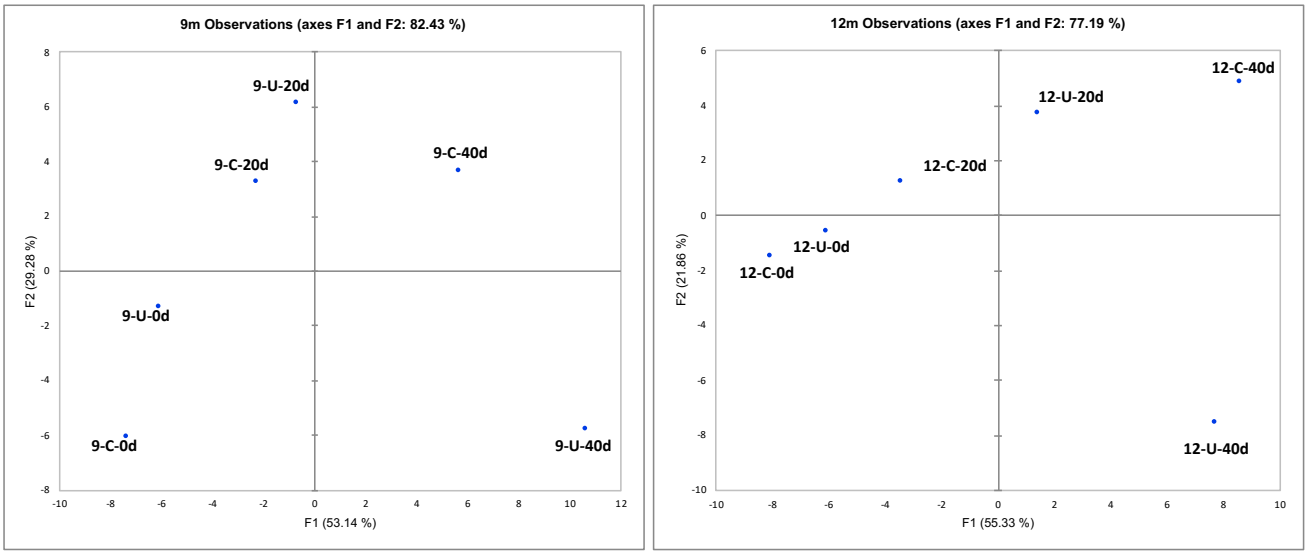

Figure 1. PCA plot of volatile compounds of 9 m and 12 m ham slices samples (C, Control; U, ultrasonic group) after refrigerated vacuum storage for 0, 20 and 40 d [5].

A total of 76 volatile compounds were identified in the 9 m and 12 m dry-cured ham samples. As shown in Figure 1, along PC1, 9 m or 12 m ham samples were distinguished by storage time with locating from left to right as the storage time progressed. Along PC2, there was no obvious separation between ultrasonic sample and control at 0 d and 20 d of storage while a distinct difference appeared at 40 d of storage. The above results indicate that ultrasound had significant influence at long vacuum storage times. Sensory results (data not shown) at the 40 d of storage of dry-cured hams revealed the ultrasound had a positive effect on pleasant odors (cured and nutty notes) which were related to the increase of 3-methyl-butanal, 2-pentyl-furan, 2-heptanone and 2-nonanone as shown in Figure 2.

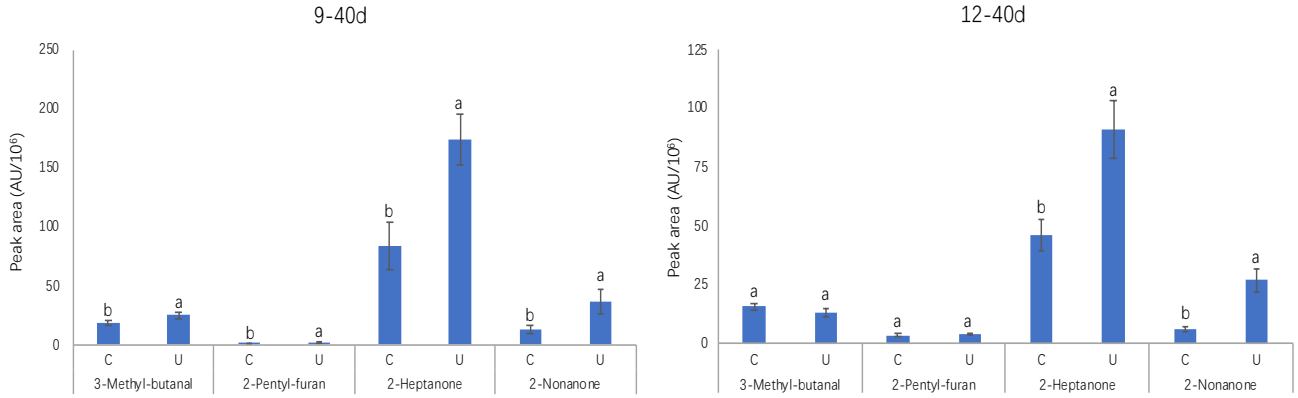

Figure 2. The peak area of volatile flavor compounds related with cured and nutty notes in 9 m and 12 m ham slices samples after refrigerated vacuum storage for 40 d (C, Control; U, ultrasonic group).

IV. CONCLUSION

The application of ultrasound to dry-cured ham slices improved the flavor characteristics during the vacuum refrigeration storage. The volatile profile differences caused by ultrasound were significantly increased in both hams and at longer storage times.

ACKNOWLEDGEMENTS

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THE USE OF ORGANIC ACID WHEY DURING COOKED SAUSAGE PRODUCTION WITH REDUCED ADDED NITRITES

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I. INTRODUCTION

In recent years, consumers are increasingly looking for foods with fewer food additives, because it is perceived to be healthier [1]. Nitrite is a key ingredient in meat curing, creating the desired sensory properties and safety of meat products but its use raises social concern. [2]. European Union legislative solutions are aimed at limiting this ingredient in meat products. A significant reduction in the amount of nitrites in the meat curing process may adversely affect the quality and durability of meats produced using traditional methods by small processors, where no other chemical additives are used to support the curing process as in the large conventional meat industry. Acid whey is a natural by-product of the production of cottage cheese that contains lactic acid, whey proteins, vitamins and compounds with antioxidant activity, i.e., glutathione and lactoferrin. Previous research showed the positive effect of acid whey on the colour, taste and shelf life length of uncured meat products [3]. Nevertheless, the properties of organic acid whey, especially the content of organic acids and antioxidant ingredients, has potential for the use in the production of meat products with reduced added nitrite that can influence the formation of desired quality features and durability of products. Therefore, this research aimed to assess the impact of using organic acid whey in the meat curing process with a reduced amount of sodium nitrite on the physicochemical properties and microbiological quality of cooked sausages.

II. MATERIALS AND METHODS

In this experiment 4 meat batter treatments were prepared for sausage production: (C1) treatment with a 2.0% nitrite curing mixture (99.5% NaCl, 0.5% $NaNO_2$), (C2) treatment with a 1.0% nitrite curing mixture and 1.0% of salt, (S) treatment with 2.0% of salt and (CW) with a 1.0% nitrite curing mixture, 1.0% of salt and 5.0% of organic acid whey (Bydgoszcz, Poland). The glucose (0.5%) was added to all treatments and water to replenish the mass of sausages. The pork trimmings meat were minced (8 mm), mixed with ingredients and left for 48 h at 4-6 °C (curing time). Next, the meat batter was stuffed into casings and the sausages were smoked (55–65 °C, 1 h) and cooked up to 70 °C in the centre. The products were chilled, vacuum-packed and cold-stored. In the samples the following values were defined: pH and redox potential using Delta 350 pH meter, thiobarbituric acid-reactive substance (TBARS) by Pikul method, colour parameters in the system CIE L*a*b* using Minolta CR-300, total viable counts (ISO 4833-2:2013), the presence of *Salmonella* spp. (ISO 6579-1:2017), *Listeria monocytogenes* (ISO 11290-2:2017) and *Clostridium* spp. (PN-A-82055–12). The experiment was performed at 3 replications (n=3) in industrial conditions. The two-way ANOVA included the main effects (treatments), and the storage period (0, 14 days) as well as their interactions were used. The Fisher's LSD test was used to determine the significance of the mean values for a multiple comparison at $P < 0.05$. The Statistica program, version 13 was used.

III. RESULTS AND DISCUSSION

The research results are presented in Table 1. The addition of acid whey did not significantly affect the pH of the sausages after production. The dynamics of pH changes during sausage storage depended on the treatment used. After production, the lowest redox potential value was found in CW treatment ($P < 0.05$), which was probably related to the antioxidant effect of acid whey components. It was also observed that in the CW treatment, the TBARS value was lower than in the C2 treatment

($P < 0.05$) and similar to that of cured sausage with normal nitrite content (C1). After storage, the significantly highest TBARS value was found in the uncured sample (S), which indicates that the amount of secondary fat oxidation products in these samples was the highest. After production and storage, the CW sausages were characterized by a higher value of a* parameter than C2 sausages ($P < 0.05$) and were similar to the C1 treatment. High redness may be related to the reductivity of the meat system with acid whey and the amount of nitrosyl pigments [3,4]. Treatments C1 and CW generally had similar brightness (L*). The S sample had the highest yellowness (b*) after production and storage. All of the tested products were free of pathogenic bacteria. After storage, the total number of bacteria in all experimental samples remained at a similar level and ranged from 10^3 to 10^4 cfu/g.

Table 1 - The pH, ORP, TBARS and colour parameters (L, a*, b*) of the experimental sausages.

Parameter	Sampling time (days)	Treatment (n=3)				S x T
		C1	C2	S	CW	P
pH	0	5.90 ± 0.03^{Aa}	5.85 ± 0.03^{Aa}	6.03 ± 0.11^{Ab}	5.86 ± 0.07^{Aa}	***
	14	6.02 ± 0.02^{Bbc}	6.11 ± 0.02^{Bc}	5.85 ± 0.11^{Aa}	5.94 ± 0.08^{Aab}	
ORP (mv)	0	428.83 ± 2.11^{Bc}	423.17 ± 2.27^{Bb}	433.33 ± 5.82^{Ac}	410.17 ± 1.77^{Aa}	***
	14	397.17 ± 0.90^{Aa}	404.17 ± 4.30^{Aa}	428.83 ± 3.72^{Ab}	433.83 ± 6.89^{Ba}	
TBARS (mg MDA/kg)	0	0.58 ± 0.01^{Aa}	0.66 ± 0.06^{Ab}	0.93 ± 0.06^{Ac}	0.55 ± 0.02^{Aa}	***
	14	0.64 ± 0.06^{Aa}	0.68 ± 0.04^{Aa}	0.85 ± 0.06^{Ab}	0.69 ± 0.05^{Ba}	
L*	0	63.52 ± 3.27^{Aab}	63.70 ± 4.34^{Aab}	65.58 ± 0.91^{Ab}	61.55 ± 3.18^{Aa}	NS
	14	62.75 ± 3.28^{Aab}	64.18 ± 2.93^{Ab}	66.87 ± 2.94^{Ac}	61.52 ± 2.88^{Aa}	
a*	0	8.56 ± 1.50^{Ac}	6.94 ± 1.80^{Ab}	3.96 ± 0.53^{Aa}	8.80 ± 1.59^{Ac}	NS
	14	8.68 ± 1.81^{Ac}	7.35 ± 2.02^{Ab}	3.71 ± 1.15^{Aa}	9.30 ± 1.69^{Ac}	
b*	0	9.02 ± 0.28^{Aa}	8.90 ± 0.38^{Aa}	10.21 ± 0.43^{Ab}	9.01 ± 0.43^{Aa}	NS
	14	8.97 ± 0.32^{Aa}	9.13 ± 0.27^{Aa}	10.38 ± 0.42^{Ab}	8.95 ± 0.49^{Aa}	

Means followed by the different lowercase letters (a-c) between the treatments at the same storage time and capital letters (A-B) between the same treatment at different storage times are significantly different ($P < 0.05$). SD: standard deviation. P: significance of effects treatment-time interaction; NS – not significant; ***$P < 0.001$.

IV. CONCLUSION

Implementing organic acid whey to the meat curing process with a reduced amount of sodium nitrite enabled a higher redness (a*) in the meat product than in the case of the product cured without acid whey. The pork sausages with acid whey were characterized by the storage-stable pink colour, oxidative and storage stability. This research shows that the suggested technology may be a promising solution to produce good quality meat products with reduced addition of sodium nitrite.

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THE INFLUENCE OF ORGANIC ACID WHEY FROM VARIOUS REGIONS IN POLAND ON THE LIPID COMPOSITION AND OXIDATIVE STABILITY OF COOKED SAUSAGE

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I. INTRODUCTION

The research conducted at IBPRS-PIB has demonstrated that the incorporation of organic acid whey into the production process positively influences the colour, quality, and storage stability of uncured meat products [1; 2]. Acid whey also has antioxidant and antibacterial properties. It has been shown that acid whey can affect the profile of fatty acids and demonstrate lipolytic activity, which may explain the increased PUFA ratio in the product [2]. Whey's composition and functional properties may vary depending on the region of production due to the diverse composition of milk. The research aimed to assess the influence of acid whey obtained from various regions in Poland on changes in the fatty acid profile, oxidative stability, physico-chemical parameters, and microbiological quality of cooked sausage.

II. MATERIALS AND METHODS

In this experiment 4 meat batter treatments were prepared for sausage production: (C) treatment with 2.0% of salt, 0.5% glucose, 5% water; (S1) treatment with 2.0% of salt, 0.5% glucose, 5% organic acid whey (Dukla, Poland); (S2) treatment with a 2.0% of salt, 0.5% glucose, 5% organic acid whey (Bydgoszcz, Poland); (S3) treatment with 2.0% of salt, 0.5% glucose and 5% of organic acid whey (Częstochowa, Poland). The pork trimmings meat were minced (8 mm), mixed with ingredients and left for 48 h at 4-6 °C. Next, the meat batter was stuffed into casings and the sausages were smoked (55–65 °C, 1 h) and cooked up to 70 °C in the centre. The products were chilled, vacuum-packed and cold stored at 4–6 °C. In the samples the following values were defined: pH and redox potential, thiobarbituric acid-reactive substance (TBARS), microbiological (*Salmonella spp.* ISO 6579-1:2017, *Listeria monocytogenes* ISO 11290-2:2017 and *Enterobacteriaceae* ISO 21528-2:2005), fatty acid profile, lipid quality indices (AI, TI) and sensory quality (QDP, ISO 13299:2016-05). The experiment was performed at 3 replications (n=3) in industrial conditions. The two-way ANOVA included the main effects (treatments), and the storage period (0, 14 days) as well as their interactions were used. The Fisher's LSD test was used to determine the significance of the mean values for a multiple comparison at $P < 0.05$. The Statistica program version 13 was used.

III. RESULTS AND DISCUSSION

After production, sausage S3 exhibited a lower pH compared to the other samples ($P < 0.001$). This variance is likely attributed to the fermentation initiated by lactic acid bacteria found in acid whey, resulting in the accumulation of lactic acid. However, after 14 days, the pH of sausage S3 increased, possibly due to the production of alkaline substances caused by proteolysis. The analysis of TBARS values revealed that sausage S3 had a significantly lower MDA content initially, but after 14 days, the C sausage showed the lowest TBARS index. The decrease in MDA content during storage is because secondary lipid oxidation products may be further degraded to other, more stable compounds [3]. Moreover, during storage, the permitted amount did not exceed the MDA content in meat products, which is approx. 2-2.5 mg/kg of product. Analysis of the ORP indicated that initially, the S2 sausages exhibited to be the most favourable (the lowest value). However, after 14 days, the S3 sausages presented the lowest ORP index, likely due to the accumulation of protein proteolysis products ($P < 0.001$). After production, all sausage treatments were generally characterized by good sensory quality. Nevertheless, it was shown that the S3 sausage was rated as juicier, of the highest overall quality and more aromatic. This observation may be related to the acidity and TBARS values of the products. Our

study also found that the addition of acid whey positively changed the fatty acid profile during storage, with an increase in the percentage of MUFA and PUFA. Lipid analysis showed that the sausage samples had low atherogenicity (AI) and thrombogenicity (TI) indices, potentially suggesting better nutritional quality of the product (Table 1). Additionally, the sausage samples exhibited good microbiological quality throughout the storage period, with no detection of *Listeria monocytogenes*, *Enterobacteriaceae* and *Staphylococcus aureus*.

Table 1 - The pH, ORP, TBARS and fatty acid of the cooked sausages (means ± SD).

Parameter	Sampling time (days)	Treatment (n=3)				Treatment x time
		C	S1	S2	S3	P
pH	0	6.01 ± 0.13^{bB}	5.99 ± 0.05^{bA}	5.98 ± 0.03^{bA}	5.89 ± 0.06^{aA}	***
	14	5.89 ± 0.13^{aA}	5.91 ± 0.07^{abA}	5.98 ± 0.05^{bcA}	6.02 ± 0.06^{cB}	
ORP [mv]	0	421.00 ± 10.34^{bA}	406.78 ± 6.53^{aA}	398.56 ± 3.59^{aA}	451.67 ± 14.43^{cB}	***
	14	424.22 ± 7.21^{cA}	405.67 ± 3.80^{bA}	406.22 ± 10.64^{bA}	395.11 ± 9.70^{aA}	
TBARS [mg MDA/kg]	0	0.95 ± 0.06^{cdA}	0.90 ± 0.06^{bA}	0.99 ± 0.04^{dA}	0.76 ± 0.05^{aA}	***
	14	0.88 ± 0.07^{aA}	0.91 ± 0.05^{abB}	1.12 ± 0.05^{dB}	0.96 ± 0.04^{cB}	
Σ UFA [%]	0	59.07 ± 0.26^{abA}	59.03 ± 0.12^{abA}	59.13 ± 0.25^{abA}	58.73 ± 0.17^{aA}	*
	14	58.83 ± 0.12^{abA}	58.97 ± 0.17^{abA}	59.67 ± 0.09^{dB}	59.20 ± 0.24^{cB}	
n-3 [%]	0	$0,90\pm0.00^{bA}$	$0,83\pm0.05^{aA}$	$0,90\pm0.00^{bA}$	$0,80\pm0.00^{aA}$	NS
	14	$0,90\pm0.00^{aA}$	$0,93\pm0.05^{aB}$	$0,93\pm0.05^{aA}$	$0,90\pm0.00^{aB}$	
n-6 [%]	0	$10,63\pm0.61^{abA}$	$10,37\pm0.25^{abA}$	$10,43\pm0.05a^{bA}$	$10,07\pm0.26^{aA}$	NS
	14	$10,27\pm0.09^{abA}$	$10,43\pm0.34^{abA}$	$10,80\pm0.24^{bA}$	$10,33\pm0.12^{abA}$	
AI	0	$0,439\pm0.005^{aA}$	$0,441\pm0.002^{bA}$	$0,437\pm0.003^{aA}$	$0,447\pm0.004^{bB}$	*
	14	$0,444\pm0.002^{bB}$	$0,442\pm0.003^{bA}$	$0,433\pm0.002^{aA}$	$0,439\pm0.003^{aA}$	
TI	0	$1,263\pm0.012^{aA}$	$1,273\pm0.009^{abA}$	$1,257\pm0.011^{aB}$	$1,292\pm0.013^{bB}$	*
	14	$1,272\pm0.008^{bcA}$	$1,264\pm0.008^{bA}$	$1,233\pm0.007^{aA}$	$1,256\pm0.010^{bA}$	

a-c: Means in the same row with different superscript small letters differ significantly ($P < 0.05$); A-B: Means in the same column with different superscript capital letters differ significantly ($P < 0.05$); UFA – the sum of monounsaturated and polyunsaturated fatty acids; n-3 – the sum of the n-3 fatty acids; n-6 – the sum of the n-6 fatty acids; The values are expressed as means ± SD. *P*: significance of effects; treatment; time; treatment-time interaction; NS – not significant; *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$. AI – Index of Atherogenicity; IT – Index of Thrombogenicity.

IV. CONCLUSION

The research indicates that the suggested technology could potentially offer a promising solution for reducing oxidative changes and enhancing the fatty acid profile of meat products. However, it should be noted that the properties of whey may vary depending on place of origin. Therefore, additional research is required to investigate this issue further.

ACKNOWLEDGEMENTS

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SESSION 11

Meat and Health

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SELENIUM, IRON, ZINC AND MAGNESIUM CONCENTRATIONS IN BRAZILIAN BEEF (*LONGISSIMUS LUMBORUM*) FROM ANGUS, WAGYU AND NELLORE CATTLE

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I. INTRODUCTION

Beef is an excellent food that provides most of the nutrients needed for a balanced and healthy diet. It contains a large amount of protein, vitamins such as A, D, E, B12 and essential minerals [1], which participate in the body's physical-chemical and maintenance processes [2]. Foods that provide insufficient amounts of nutrients have poor nutritional quality and are linked to various forms of malnutrition around the world, eating nutritious food is essential for good health and disease prevention [3]. The amount of minerals in beef can vary between different breeds [4], however, there are few studies in Brazil on the variation in mineral content available in beef, and the influence that different breeds can have on this process. For these reasons, the aim of this study was to evaluate the concentrations of iron, zinc, magnesium, and selenium in beef from the Angus, Wagyu and Nellore breeds.

II. MATERIALS AND METHODS

The meat used in the study was obtained from cattle Angus (n=5) and Wagyu (n=5), were raised in the countryside of São Paulo state, under tropical savannah climate conditions. Upon reaching 17 months of age, the cattle were transitioned to a feedlot system where they were provided with a specialized fattening diet. Prior to slaughter, the average weight of Angus was 639kg, and Wagyu 755kg. Each sample was meticulously tracked from the point of slaughter up to the boning process, allowing for a direct correlation with their respective source animals and the cuts. As for the Nellore samples (n=3), they were acquired in whole cut at a local butcher shop. This experiment utilized a total of 13 sirloin samples (Longissimus lumborum). The minerals iron, zinc and magnesium were quantified by Flame Atomic Absorption Spectrophotometry (FAAS - Perkin Elmer, USA, model AAnalyst-200), following the method described by Rebellato et al [5]. To assess selenium, the samples were subjected to acid digestion and incineration at 450°C to detect the mineral by Hydride Generation Atomic Absorption Spectrometry (HG-AAS), following the method described by Orlando et al. [6]. The data obtained was analyzed with one-way ANOVA and Tukey's test ($P<0.05$), using Statistica v.10 software (StatSoft, USA).

III. RESULTS AND DISCUSSION

The concentrations of the minerals evaluated in the three breeds are shown in Table 1, along with the reference values available in the Brazilian Table of Food Composition - TBCA [7] for the same cut (*Longissimus lumborum*, fat-free and raw). The Wagyu and Nellore samples did not differ significantly in terms of iron content, higher values than Angus ($P<0.05$). Based on the Recommended Daily Intake (RDI) for adults, published in resolution No269/2005 [8] by the Brazilian Ministry of Health/ANVISA, a new value was calculated for the amount of iron, considering the higher absorption rate of heme iron in meats. The calculated RDI for heme iron was 4.2mg, so just one 200g (raw) Wagyu steak could supply more than the daily iron requirement.

Table 1. Means* of iron, zinc, magnesium and selenium concentrations determined in Angus, Wagyu and Nellore beef tenderloin (*Longissimus lumborum*). Results expressed as mean ± standard deviation.

Breed	Iron (mg/100g)	Zinc (mg/100g)	Magnesium (mg/100g)	Selenium (µg/100g)
Angus	$1.30^{b} \pm 1.16$	$4.12^{a} \pm 2.75$	$26.04^{a} \pm 3.83$	$8.87^{b} \pm 0.42$
Wagyu	$2.17^{a} \pm 1.55$	$4.25^{a} \pm 2.48$	$21.31^{b} \pm 7.57$	$11.29^{a} \pm 0.94$
Nellore	$2.03^{a} \pm 0.89$	$3.83^{a} \pm 4.03$	$25.27^{a} \pm 9.81$	$6.61^{c} \pm 0.36$
Reference**	1.68	3.36	19.6	2.87
RDI***	4.2****	7	260	34

*Equal letters in the same column for the same mineral do not differ significantly in Longissimus dorsi according to Tukey's test of means (P<0.05). **Average concentrations of Fe, Zn, Mg and Se for the same cut, according to the Brazilian Table of Food Composition - TBCA [7]. ***Recommended Daily Intake [8]. ****Adjusted value for iron-heme in meat.

It was found that there was no difference in magnesium concentrations between Angus and Nellore samples, but both had higher values when compared to Wagyu (P<0.05). Compared to the RDI of 260mg, the magnesium concentrations show that beef is a poor source. There was no significant difference in the amounts of zinc found in the samples of the three breeds evaluated (P<0.05). The results show that the samples of the three breeds are good sources of zinc in relation to the RDI, which recommends 7mg. Wagyu beef had the highest selenium concentration, followed by Angus beef, and the Nellore values were the lowest among the three breeds (P<0.05). Considering that the RDI for selenium is 35µg, it can be seen that Wagyu beef can provide a good amount of this mineral. According to the reference values [8], it can be seen that the Angus samples have lower iron concentrations, and in Nellore the selenium results were lower than the three races (P<0.05). Samples from all three breeds had zinc and magnesium concentrations above the results in TBCA. For the four minerals evaluated, the Wagyu samples showed higher values than the averages published in the TBCA for the same cut.

IV. CONCLUSION

The results can be used in the areas of nutrition and research related to the nutritional content of meat. The availability of nutrients, especially minerals, can be exploited in the way products are presented to the consumer, with more nutritionally attractive meats.

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POLYSACCHARIDE HYDROGELS WITH OLIVE OIL TO DEVELOP SUSTAINABLE MEAT PRODUCTS WITH A HEALTHIER LIPID CONTENT

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I. INTRODUCTION

The meat sector is currently facing complex challenges, defined by the social requirements of safety, quality and sustainability and a consumer who demands healthier products. In this regard, different strategies have been proposed for incorporation of healthy oils in a gel-like matrix to form oil bulking agents with potential to use as animal fat replacers in meat products [1]. These would contribute to reducing the use of raw materials of animal origin, as part of the current interest in finding new product formulations that meet societal concerns. The aim of this work was to evaluate composition and technological and structural properties, of frankfurters formulated using polysaccharide hydrogels with olive oil as animal fat replacers.

II. MATERIALS AND METHODS

Four different samples were elaborated (Table 1): two controls with pork backfat (PF), normal-fat (NPF) and low-fat (LPF) and two reformulated in which the PF was completely replaced by polysaccharides hydrogels with olive oil (HO) to prepared normal (NHO) and low fat (LHO) frankfurters [2]. Additives added: 2.0 g/100 g NaCl; 0.30 g/100 g sodium tripolyphosphate; 0.012 g/100 g sodium nitrite; and 0.5 g/100g flavouring. HOs were prepared mixing in a homogenizer sodium alginate, $CaSO_4$, sodium pyrophosphate and inulin (2.25%) with water and olive oil [3]. Composition (moisture, ash, protein and fat), technological properties (processing loss (PL), pH, hardness and color) and structural characteristic, using FT-Raman spectroscopic, were determined in all frankfurters [2, 4]. Results were evaluated by ANOVA followed by Tukey's test ($p<0.05$).

Table 1- Formulation [% meat, pork backfat (PF), polysaccharides hydrogels with olive oil (HO), and water] of frankfurters.

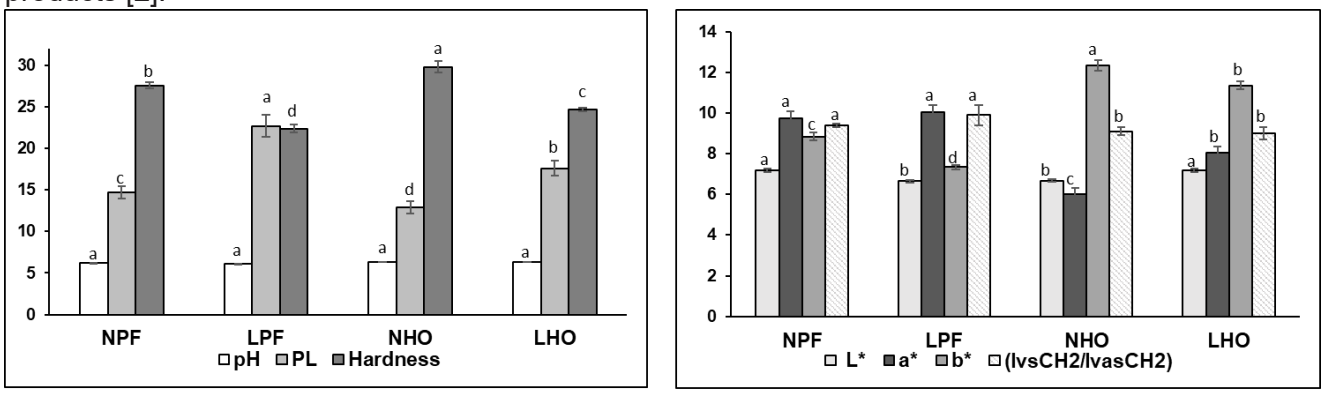
Samples*	Meat (%)	PF (%)	HO (%)	Water (%)
NPF	63	21	-	13.19
LPF	63	9	-	25.19
NHO	63	-	32.5	1.69
LHO	63	-	14.0	20.19

*formulated with normal (NPF) and low (LPF) por backfat (PF) content and with polysaccharides hydrogels with olive oil (HO) used as total PF replacers in normal (NHO) and low (LHO) fat content samples. Means ± standard deviation. Different letters in the same column indicate significant differences ($P<0.05$).

III. RESULTS AND DISCUSSION

Moisture ranged between 60.5 to 71.9% and the highest ($p<0.05$) values were observed in low-fat frankfurters (LPF and LHO) according to the formulation (Table 1). Ash contents varied between 2.79 and 4.42%, of which the frankfurters with HO (NHO and LHO) displayed the highest ($p<0.05$) values. Protein content was similar ($p>0.05$) in all frankfurters. Fat content ranged between 9.8 and 20.4%, and the highest ($p<0.05$) values were recorded in normal-fat frankfurters (NPF and NHO) according to formulation (Table 1). All frankfurters showed similar ($p>0.05$) pH values (Figure 1A). When compared,

samples with similar level of fat to those reformulated with HO presented lower ($p<0.05$) PL and higher ($p<0.05$) hardness (Figure 1A). The lowest ($p<0.05$) a* values corresponded to samples with HO (NHO and LHO), while these frankfurters showed the highest ($p<0.05$) b* values. These findings could be attributed to NHO and LHO containing the lowest amount of meat material and highest amount of olive oil, which are related to a more pronounced yellow color. Analysis of the 2800–3025 cm^{-1} region from Raman spectra showed that the lowest $Iv_sCH_2/Iv_{as}CH_2$ value (I2850/I2880 cm^{-1}) (Figure 1B) corresponded to samples reformulated with OH (NHO and LHO). These results indicated more lipid acyl chain disorder which suggests more lipid-protein interactions in frankfurters elaborated with HO as pork backfat replacer. A significant correlation was found between processing loss, hardness and this specific structure. These results are in concordance with previous findings obtained in similar meat products [2].



(A) (B)

Figure 1. (A) pH values, processing loss (PL) and hardness (B) color parameters (L*/10, a*, b*) and $Iv_{as}CH_2/Iv_sCH_3$ /10 relative intensity from Raman spectra frankfurters. See Table 1 for sample denomination. Different letters in each parameter indicate significant differences ($p<0.05$).

IV. CONCLUSION

The strategy based on using polysaccharide hydrogels with olive oil as an animal fat replacer in frankfurters simultaneously improved their lipid content and reduced the use of meat raw materials, in line with current trends. Besides, this reformulation procedure does not negatively affect technological properties of the final meat product. On the other hand, an interesting relationship was found between protein and lipid interactions and specific technological properties such as texture or processing loss.

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EFFECT OF HEMOGLOBIN DEGRADATION ON THE ZINC PROTOPORPHYRIN IX FORMATION IN PORK LIVER HOMOGENATE

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I. INTRODUCTION

Zinc protoporphyrin IX (ZnPP) contributes to the bright red color of Parma ham, a traditional Italian dry-cured ham prepared without nitrite/nitrate [1]. ZnPP is endogenously formed in meat and internal organs. Among the different internal organs, the liver shows an outstanding ZnPP-forming capacity [2]; thus, it could be applied for color improvement of nitrite/nitrate-free meat products. However, the reason for the high ZnPP-forming capacity in the liver remains unclear. The optimum pH of ZnPP formation in the liver was reported to be relatively low, around 4.5–5.0 [2,3]. In addition, some skeletal muscles rich in slow-twitch muscle fibers show high ZnPP formation and low optimum pH (4.75) [4]. Because heme is the precursor of ZnPP, the release of heme induced by myoglobin (Mb) degradation at a lower pH is suggested to contribute to high ZnPP formation in these muscles. Thus, it can be hypothesized that hemoglobin (Hb), the main heme protein in the liver, is degraded during ZnPP formation, contributing to its high ZnPP-forming capacity. Therefore, this study aimed to elucidate the effect of Hb degradation on ZnPP formation in liver homogenate.

II. MATERIALS AND METHODS

As a model experimental system for ZnPP formation, 20% liver homogenate was supplemented with antibiotics, adjusted to pH 4.5, and then incubated anaerobically at 25°C for 3 days. For quantification, heme and ZnPP were extracted using an ethyl acetate-acetic acid mixture (4:1) and separated using reversed-phase high-performance liquid chromatography. In addition, the amount of ZnPP formed under various conditions was evaluated based on the fluorescence intensity (excitation/emission: 420/590 nm) of the 75% acetone extract of the model homogenate. EDTA, carbon monoxide (CO), and pepstatin A were added to the model homogenate as a divalent metal ion chelator, a ligand that stabilized heme iron, and a protease inhibitor, respectively. Hb degradation was evaluated by western blotting (WB) method. Statistical analysis was performed using a one-way analysis of variance with Tukey's honest significant difference test. $P < 0.05$ was considered as statistically significant.

III. RESULTS AND DISCUSSION

The ZnPP formation pathway was investigated in liver homogenates. During incubation, the heme levels decreased with an increase in ZnPP. The addition of EDTA inhibited ZnPP formation and led to the accumulation of protoporphyrin IX (PPIX). These results indicate that ZnPP in the liver homogenate is formed from heme via PPIX in the same way as in the skeletal muscle [5]. In addition, CO significantly inhibited ZnPP formation, suggesting that the release of heme from heme proteins is essential for ZnPP formation. Because Hb is the most abundant heme protein in the liver, it is assumed to contribute to ZnPP formation as a heme donor.

Next, Hb degradation during the incubation was evaluated using WB. On day 1, the band density of the Hb β chain drastically decreased (Fig. 1A), indicating that a large part of Hb was degraded at an early period of incubation. In addition, Hb degradation was vigorous in the lower pH, compared to the

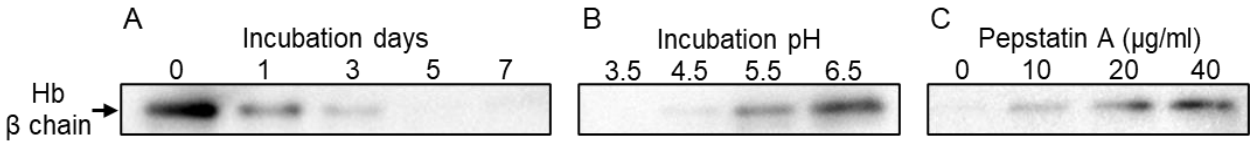

Figure 1. WB images of the model homogenate with different incubation periods (A), pH (B), and concentration of pepstatin A added (C). A and C were incubated at pH 4.5, and B and C were incubated for 3 days.

neutral pH (Fig. 1B), suggesting the involvement of acid proteases. Moreover, pepstatin A, which is the competitive inhibitor of the aspartic proteases, inhibited the Hb degradation dose-dependently (Fig. 1C). Thus, the aspartic proteases such as cathepsin D and E were suggested to degrade Hb during the incubation, especially in the early period. However, pepstatin A did not affect the ZnPP formation (Fig. 2). For the reason, the high ability of Hb to dissociate heme without degradation, which is reported to contribute fast ZnPP formation in meat products [6] could be considered. Consequently, in the liver at low pH, Hb was degraded during the incubation and thus in turn lead to heme release. However, this degradation would not be essential for the high ZnPP formation amount of the liver homogenate.

Conversely, nevertheless its high ZnPP content, the appearance of the liver homogenate after ZnPP formed is inferior to that of the skeletal muscle. Because ZnPP formed in skeletal muscle binds to apo-Mb and apo-Hb for solubilization [5], Hb degradation in the liver homogenate might affect the solubility of ZnPP and, its appearance. In this study, pepstatin A addition improved the appearance of the homogenate compared to the group without pepstatin A (Fig. 3), suggesting that Hb degradation caused an undesirable color.

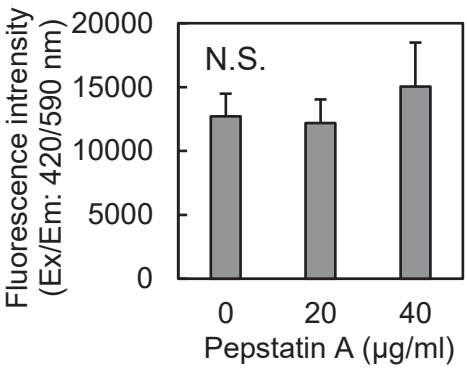

Figure 2. The effect of the addition of pepstatin A on the ZnPP formation in the liver homogenate. Bars: standard errors (n = 6). N.S.: no significant difference among the groups.

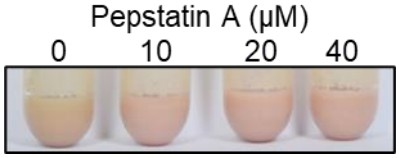

Figure 3. The appearance of the liver homogenate after incubated with different concentrations of pepstatin A for 3 days.

IV. CONCLUSION

It has been suggested that ZnPP is formed from heme dissociated from heme proteins, mainly Hb, in the liver, as in the skeletal muscles. Hb degradation during incubation was confirmed but was not essential for high ZnPP formation. Moreover, Hb degradation has been suggested to result in an undesirable appearance. Therefore, the prevention of Hb degradation was assumed to improve the coloring effect of the liver homogenate without compromising its high ZnPP-forming capacity.

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ANGUS AND WAGYU BEEF: ESSENTIAL MINERALS, FROM DIFFERENT CUTS

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1. INTRODUCTION

The skeletal muscles of animals have predetermined anatomical and structural characteristics, primarily related to their function and location in the animal's body [1]. Variations in the physicochemical composition of meats from the same animal can occur depending on the muscle groups from which they are obtained [2], potentially influencing the concentrations of elements available for absorption through the digestion process. Beef, an excellent food source, can provide most of the nutrients needed for maintaining good health, such as essential minerals like iron (Fe), zinc (Zn), magnesium (Mg), and selenium (Se), wich are easily absorbed during meat digestion [3]. Fluctuations in mineral levels can impact the sensory and nutritional quality of meats [4]. While existing literature covers discrepancies in breeds and cuts of beef, no studies have explored the racial impact and nutrient concentrations across different cuts from the same animal. Therefore, this study aimed to evaluate the protein and fat content, as well as the concentrations of iron, zinc, magnesium, and selenium in beef sourced from three cuts of meat from Angus and Wagyu breeds.

1. MATERIALS AND METHODS

A total of 10 animals were used in the experiment, all raised for beef in a geographical region characterized by a tropical savannah climate. When they reached 17 months of age, they were placed in confinement and given special feed for periods of between 105 and 266 days to fatten them up until slaughter. 5 Angus and 5 Wagyu animals were used, weighing an average of 639kg and 755kg respectively. The carcasses were identified individually until boning, so that the cuts removed could be related to their respective animals of origin. The three commercial cuts were outside flat (Biceps femoris), knuckle (Vastus intermedius, lateralis, medialis and Rectus femoris), and the shank (digital flexors and extensors). The minerals iron, zinc and magnesium were quantified by Flame Atomic Absorption Spectrophotometry (FAAS - Perkin Elmer, USA, model AAnalyst-200), following the method described by Rebellato et al [5]. To assess selenium, the samples were subjected to acid digestion and incineration at 450°C, to detect the mineral by Hydride Generation Atomic Absorption Spectrometry (HG-AAS), following the method described by Orlando et al. [6]. Protein and intramuscular fat were assessed using the Kjeldahl and Bligh & Dyer methods, respectively. The data obtained was analyzed with one-way ANOVA and Tukey's test ($P<0.05$), using Statistica v.10 software (StatSoft, USA).

1. RESULTS AND DISCUSSION

The concentrations of the four minerals examined in the three cuts are presented in Table 1, alongside the reference values for the corresponding cuts from the Brazilian Table of Food Composition - TBCA [7]. Iron levels in the knuckle and selenium levels in the outside flat and shank were notably higher in Wagyu meat ($P<0.05$). In terms of zinc and magnesium values across the three cuts, there was no distinction between Angus and Wagyu breeds ($P<0.05$). The concentrations of all four minerals in the two breeds were observed to surpass the TBCA averages, except for iron in Angus knuckle and shank ($P<0.05$). The quantities of protein and intramuscular fat are detailed in Table 2. These results were derived from the centesimal composition analyses conducted on the samples. Protein content was higher in Angus for the hard loin and rear muscle, while there was no major contrast in protein levels for the knuckle samples of the two breeds. Wagyu exhibited higher amounts of intramuscular fat in the outside flat and shank ($P<0.05$).

Table 1 – Means* ± standard deviation of the iron, zinc, magnesium, and selenium concentrations determined in beef from outside flat, knuckle and shank of the Angus and Wagyu breeds.

Cut	Breed	Iron (mg/100g)	Zinc (mg/100g)	Magnesium (mg/100g)	Selenium (µg/100g)
Outside Flat	Angus	2.01^{a} ± 1.65	3.61^{a} ± 0.67	23.78^{a} ± 8.77	8.16^{b} ± 5.60
	Wagyu	2.38^{a} ± 2.05	3.8^{a} ± 3.11	21.93^{a} ± 11.21	10.27^{a} ± 3.39
	Ref.**	1.89	2.81	21.1	2.87
Knuckle	Angus	1.71^{b} ± 0.57	5.55^{a} ± 3.88	22.96^{a} ± 4.70	10.53^{a} ± 3.99
	Wagyu	2.71^{a} ± 0.10	5.38^{a} ± 2.67	21.4^{a} ± 10.99	11.52^{a} ± 3.67
	Ref.**	1.78	4.31	21.2	2.22
Shank	Angus	1.67^{a} ± 0.80	4.87^{a} ± 0.44	22.26^{a} ± 4.73	3.73^{b} ± 2.92
	Wagyu	2.29^{a} ± 2.98	5.09^{a} ± 3.66	21.85^{a} ± 10.84	5.14^{a} ± 2.15
	Ref.**	1.86	3.65	17.5	2.24

*Means with equal letters in the same column for the same cut do not differ significantly according to Tukey's test of means (P<0.05). **Concentrations of Fe, Zn, Mg and Se for the same cut, available from TBCA [7].

Table 2 – Means* ± standard deviation of protein and intramuscular fat determined in beef from knuckle, outside flat and shank of the Angus and Wagyu breeds.

Cut	Breed	Protein (%)	I.M. Fat (%)
Outside Flat	Angus	20.97^{a} ± 0.51	6.39^{b} ± 1.20
	Wagyu	18.25^{b} ± 0.46	14.99^{a} ± 2.03
Knuckle	Angus	20.77^{a} ± 0.04	4.51^{a} ± 0.55
	Wagyu	20.03^{a} ± 0.70	7.20^{a} ± 1.64
Shank	Angus	22.73^{a} ± 0.13	4.06^{b} ± 0.47
	Wagyu	21.08^{b} ± 0.57	8.03^{a} ± 1.06

*Means with equal letters in the same column for the same cut do not differ significantly according to the ANOVA test (P<0.05).

1. CONCLUSION

The results are of significant relevance to the fields of meat research, production, and technology. These data also appeal to consumers, given their interest in nutritional matters, and can be utilized on packaging and within the specialized meat market.

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ASSESSMENT OF THE NUTRITIONAL AND QUALITY IMPACT OF ADDING PITAHAYA (*Hylocereus ocamponis*) PULP FLOUR TO FRANKFURT SAUSAGES

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I. INTRODUCTION

Frankfurt-type sausage is a popular processed meat. This product, as well as other processed meat products, have been associated with the development of certain non-communicable diseases potentially explained by the high-fat content (15-30%) and quality of these fats (mostly saturated fatty acids). The addition of co-products derived from fruits and vegetables is a viable alternative to reduce the alleged harmful effects on the human health of this type of product. This is often ascribed to the protective properties of dietary fiber, whose components, have been widely demonstrated to be capable of limiting fats and sugars absorption. Pitahaya, pitaya, or dragon fruit is a tropical and subtropical fruit belonging to the Cactaceae family. The pulp of this fruit, which may be white or red, shows a high content of phytochemicals, including (poly)phenolic compounds, betalains, and dietary fiber. With the appropriate industrial treatment, it is possible to obtain potential ingredients to be used in the food industry. Therefore, this study aimed to use the flour obtained from pitahaya pulp in the formulation of Frankfurt-type sausages and determine its effect on the chemical and technological properties of these products.

II. MATERIALS AND METHODS

Frankfurt-type sausages were made following a conventional formulation [1]. Three different formulations were prepared: the original formula was used as control sample (CS). The other samples were formulated by adding 1.5% (FPPF1.5) and 3% (FPPF3) of lyophilized pitahaya pulp flour (PPF). Chemical composition and residual nitrite level (mg $NaNO_2$/kg sample) were determined according to AOAC methodology [2]. The pH values were assessed using a penetration probe, at different sites of the sample, connected to a pH-meter. The emulsion stability was evaluated as the percentage of total expressible fluid (TEF) [3] while the color parameter was analyzed in the CIEL*a*b* space. Data analysis was performed using a one-way ANOVA test and differences were considered significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

Table 1 shows the chemical composition of Frankfurt-type sausage added with PPF. The protein and fat content did not present significant differences ($p > 0.05$) between the control sample and the samples with added PPF. The moisture and ash content showed a slight decrease ($p < 0.05$) in samples with added PPF with respect to CS. Regards the residual nitrite content, as the concentration with added PPF increased, the residual nitrite levels decreased and showed significant differences ($p < 0.05$) with respect to CS. This reduction could be due to an interaction of nitrite with the different bioactive compounds present in PPF, mainly flavonoids, and betalains.

Table 1 – Chemical composition of Frankfurt-type sausage added with pitahaya pulp flour

	Moisture	Protein	Fat	Ash	Residual $NaNO_2$
CS	35.65 ± 0.09^{b}	15.87 ± 0.17	13.81 ± 0.14	1.70 ± 0.09^{a}	35.65 ± 0.89^{a}
FPPF1.5	34.80 ± 0.16^{a}	16.23 ± 0.44	12.72 ± 0.14	1.63 ± 0.08^{ab}	31.23 ± 1.09^{b}
FPPF3.0	34.89 ± 0.38^{a}	16.27 ± 0.13	13.40 ± 1.03	1.51 ± 0.03^{b}	28.39 ± 0.96^{c}
ANOVA	*	n.s.	n.s.	*	*

Values expressed as g/100 sample. All data are presented as means ± standard error. Asterisks indicate significance at * $p < 0.05$; n.s. not significant. Values followed by same letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

The pH, the emulsion stability, and CIELAB color coordinates values of Frankfurt-type sausage are shown in Table 2. The addition of PPF had no effect ($p > 0.05$) on pH values. In the same way, the emulsion stability reported as total expressible fluid (TEF %) was not affected by the addition of PPF. However, all color parameters were deeply affected ($p< 0.05$) by the use of PPF as an ingredient and this occurred in a concentration-dependent manner. Thus, the L* and b* parameters decreased the values whilst the a* increased. This result was expected because the pulp of the pitahaya shows a reddish color due to the presence of betalains and anthocyanins in its composition.

Table 2 –Physico-chemical properties of Frankfurt-type sausage with added pitahaya pulp flour.

			Color parameters		
	pH	TEF (%)	L*	a*	b*
CS	5.92 ± 0.07	1.20 ± 0.12	69.71 ± 0.14^{a}	4.32 ± 0.23^{c}	8.85 ± 0.49^{a}
FPPF1.5	5.99 ± 0.01	1.39 ± 0.11	66.28 ± 0.25^{b}	10.38 ± 0.62	6.38 ± 0.59^{b}
FPPF3.0	5.99 ± 0.04	1.48 ± 0.21	63.70 ± 0.13^{c}	13.20 ± 0.51	6.13 ± 0.19^{b}
ANOVA	n.s.	n.s	*	*	n.s.

All data are presented as means ± standard error. Asterisks indicate significance at * $p < 0.05$; n.s. not significant. Values followed by same letter in the same column were significantly different according to Tukey's HSD post-hoc test ($p < 0.05$).

IV. CONCLUSION

The addition of pitahaya pulp flour seems to be a technologically viable alternative for elaborating emulsified cooked cured meat products, such as Frankfurt-type sausages. In addition, pitahaya pulp flour could have a great potential to be used in the meat industry as an ingredient for reducing the residual nitrite levels and could improve the "natural" and "healthy" image of these products, among consumers.

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Influence of Drylot feeding at Different Altitudes on Slaughtering Performance and Meat Nutrients Composition of Yaks

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I. INTRODUCTION

Yak is the primary plateau livestock distributed on the Qinghai-Tibet Plateau, with a population of approximately 14.45 million (2022), which constitutes the pillar industry in the high-altitude areas of China. The yak industry plays a vital role in the economic and social development of the Qinghai-Tibet Plateau. However, due to the traditional grazing feeding pattern and the seasonal imbalance of feed supply, overgrazing, long raising cycle and poor meat quality of yaks continue to be inevitable issues in the industry. To address the challenges, studies have been carried out to explore the practice of transferring yaks from the Qinghai-Tibet Plateau to lower-altitude plains with drylot feeding pattern, in consideration of reducing grazing pressure of pasture, utilization of feed resources in the plain region and improving feeding efficiency. In this study, the growth, slaughtering performance, and meat quality of stall-fed yaks at different altitudes were compared to evaluate the feasibility of low altitude drylot feeding for yaks.

II. MATERIALS AND METHODS

Approximately 18 months old male yaks were selected for the experiment, which was conducted in Guanghan(low altitude 600 m, LA, n=6). The ratio of concentrate to roughage was 5:5. Yaks (n=6) with a natural grazing system in Hongyuan City (high altitude 3500 m, HA) were chosen as the control group. At the end of the experiment, LA and HA groups animals were humanely slaughtered and phenotypic data on carcass weight. The longissimus dorsi samples were transported to a refrigerator (−20 °C) in the laboratory to measure meat quality.

Table 1: Time, Altitude, and Temperature of experimental Areas

Region	Altitude (m)	Numbers of Animals (n)	Extreme High Temp (℃)	Extreme Low Temp (℃)	Average Temp (℃)
LA	600	6	36	-1	14.5
HA	3500	6	24	-22	-0.7

III. RESULTS AND DISCUSSION

（1）Growth and Slaughter Performance

Yaks in the HA group showed the slowest growth with an ADG of 145 g/d. Yak in the LA and HA groups were slaughtered at body weights of 193 kg and 152kg, respectively. The results also revealed greater carcass weight, net meat weight, and dressing percentage in the LA group compared to the HA group(Table 2).

Table 2: Growth and Slaughter Performance of Yaks at Different Altitudes

Group[1]	Initial Weight (kg)	Final Weight (kg)	Average Daily Gain (g)	Carcass Weight (kg)	Net Meat Weight (kg)	Dressing Percentage (%)
LA	130.83±35.75	193.33±40.41	268.24±42.86[a]	90.24±23.22[a]	71.25±18.44[a]	46.39±2.32[a]

HA	118.83±17.00	152.67±22.70	145.21±28.82[b]	59.38±22.49[b]	45.08±18.62[b]	42.51±2.63[b]

[1] Values in the same column with different letter superscripts differed significantly (P<0.05).

（2）Meat nutrient composition

The fat content of LA yak meat was significantly higher than that of the control group(HA). Moisture 、Ash and Protein no significant difference was observed in the comparison between LA and HA groups.

Table 3: Analysis of Meat Composition in Stall-Fed Yak

Group[1]	Moisture (%)	Ash (%)	Fat (g/100g)	Protein (g/100g)
LA	74.10±1.59	1.63±0.12	3.57±3.15[a]	22.37±0.61
HA	74.47±1.16	1.73±0.06	1.63±0.31[b]	23.07±1.21

[1] Values in the same column with different letter superscripts differed significantly (P<0.05).

（3）Meat quality of yaks at different altitudes

The IMF content and Inosine monophosphate of LA yak meat were significantly higher than that of the HA group; however, no significant differences were observed in other indicators between the LA and HA group.

Table 4: Quality analysis of yak meat for consumption

Index[1]		LA	HA
pH	pH45min	6.46±0.13	6.48±0.42
Colour difference	Brightness (L*)	31.43±4.31	32.95±2.58
	Redness (a*)	9.78±1.98	11.18±3.77
	Yellowness (b*)	6.97±1.67	9.00±1.76
Tenderness index	Cooked meat rate (%)	68.29±1.64	68.09±1.07
	Shear force (N)	5.57±0.74	7.51±2.42
	Intramuscular fat (IMF, %)	2.07±2.15[a]	0.41±0.05[b]
	Inosine monophosphate (IMP, g·kg-1)	0.55±0.07[a]	0.33±0.42[b]

[1] Values in the same row with different letter superscripts differed significantly(P<0.05).

The comparison of amino acid composition between the LA and HA groups did not show significant differences.

Table 5: Amino Acid Composition Analysis

Group[2]	TAA[1]	FAA	EAA/NEAA	FAA/TAA
LA	21.20±0.67	9.46±0.27	0.66±0.02	0.45±0.01
HA	21.43±1.30	9.52±0.53	0.66±0.01	0.44±0.01

[1]TAA=Total amino acids, FAA=Fresh amino acids, EAA/NEAA=essential/non-essential amino acids, FAA/TAAFresh amino acids/total amino acids

[2] Values in the same column with different letter superscripts differed significantly (P<0.05).

IV. CONCLUSION

In comparison with significant heat loss and forage deficiency during winter at high altitude area, the growth and slaughter performance of yaks can be enhanced by drylot feeding at lower altitude, which is also beneficial to the improvement of meat quality (Intramuscular fat, Shear force).

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THE IMPACT OF DIETARY ANSERINE ON LIPID OXIDATION DURING IN VITRO DIGESTION OF COOKED GROUND CHICKEN MEAT

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I. INTRODUCTION

Imidazole dipeptides are naturally occurring in skeletal muscles of vertebrates, of which two of the most common are carnosine (β-alanyl-L-histidine) and its derivative, anserine (β-alanyl-1-methylhistidine) [1]. Studies using the isolated molecule have demonstrated that carnosine could play a protective role in a wide range of oxidative stress-related diseases, including neurodegenerative, cardiovascular, diabetes and cancer [1,2,3,]. Recently, carnosine benefits have also been demonstrated through its ingestion in red meat-based meal models [5,6,7], showing concentration dependent protein and lipid antioxidant effects during digestion of pork. Given that anserine is found in higher concentrations in white than red meat, the objective of this study was to determine the effect of anserine on malonaldehyde, a lipid oxidation indicator, during the digestion of chicken.

II. MATERIALS AND METHODS

Meat preparation: Three levels of anserine (control, LAns; intermediate, MAns; high, HAns) were achieved by adding 300 or 600 mg anserine/100g meat to commercial ground lean chicken. The chicken was stuffed into polypropylene wide-mouth screw-top containers (60 ml), individually vacuum packaged and cooked in a water bath (core temperature 74°C). The cooked meat was vacuum packaged and stored at -80°C. Just prior to digestion, the meats were thawed at room temperature (approximately 15 min).

In vitro digestion: In vitro digestion was undertaken according to Li et al. [4]. Tubes containing cooked meat samples (6.0 g) were sequentially incubated for 5 min with saliva (6 ml), 2 h with gastric juice (12 ml), and 2 h with 1 M bicarbonate buffer (pH 8.0, 2 ml), duodenal juice (12 ml) and bile (6 ml). Enzymatic incubation was performed in quadruplicate. Digests were homogenized using a Polytron homogenizer (10,000 rpm for 1 min), transferred to Eppendorf tubes and stored at -80°C until analyses.

Lipid oxidation: Malondialdehyde (MDA) was measured by GC-MS [5] using an Agilent 7890B gas chromatograph coupled to a 5977B quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with Ultimate Plus deactivated fused silica tubing and HP-5MS columns (30 m × 0.25 mm × 0.25 µm). Selected-ion monitoring (SIM) was used (187 and 203 m/z, retention time (RT) 12.20 and 12.33 min, internal standard, HHE-d3; 250 m/z, RT 13.76 and 13.79 min, MDA) for quantification.

Statistical analyses: Results were analyzed by the MIXED model of SAS.

III. RESULTS AND DISCUSSION

Anserine showed anti-oxidant properties in gastric digests, but no significant differences were observed in the duodenal phase. In the gastric phase, anserine-enriching treatments (MAns and HAns)

resulted in significantly less MDA in digests, than the control (LAns) digests (P>0.05; Table 1). In addition, concentrations of MDA in the HAns digests were significantly lower than in the MAns digests (P>0.05) showing a progressive decrease in lipid oxidation with increased anserine concentration.

Similarly, high levels of carnosine enrichment of pork resulted in significantly lower MDA concentrations in gastric digests than at intrinsic levels of carnosine, while no effects were observed in the duodenal digests [7]. Given the range of antioxidant effects reported for carnosine, this glimpse into the potential of anserine ingestion as an antioxidant demonstrates that further research on the impact of anserine as an antioxidant is merited.

Table 1. Effects of anserine on MDA during in vitro digestion of chicken (LS Means with SEM in parentheses)

	MDA[1] (ng/ml digest)	
Treatments	Gastric	Duodenal
LAns	2727[a] (124)	2274 (86)
MAns	2218[b] (124)	2236 (86)
HAns	1826[c] (124)	2261 (86)
P values	**0.0003**	0.9476

[1] Different superscripts within the same column denote significant differences (P≤0.05).

IV. CONCLUSION

In this study, dietary anserine, which is relatively abundant in white meat, demonstrated antioxidant capacity during the digestion of chicken. These findings suggest that dietary anserine may bring similar health benefits through its ingestion in a meal as carnosine, from which it is derived, imparted in red meat-based meal models.

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PROBIOTIC POWDER IN A PEMMICAN MODEL

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I. INTRODUCTION

Pemmican is a meat product principally comprised of dried meat and fat that has a played a significant role throughout history as a source of sustenance in cold, harsh climates for some Indigenous peoples, fur traders, polar explorers, military and police [1]. Acclaimed for its high nutrient density and long shelf life, pemmican shows potential as a culturally appropriate food for use as emergency provisions to alleviate periods of food insecurity or in crisis situations. Furthermore, given its high proportion of fat (up to 50%), pemmican appears a good vector for the delivery of probiotic bacteria as demonstrated in cocoa butter [2] allowing for added health benefits to an emergency provision. The objective of this study was therefore to determine the stability of probiotic bacteria in a pemmican model.

II. MATERIALS AND METHODS

Meat processing: Commercial vacuum-packaged frozen pork loins were thawed at room temperature (RT) for 21 h. Longissimus dorsi (LD) lean was isolated, minced (batches of about 25 kg; 3.5 mm plate hole diameter) and spread onto stainless steel trays (45.5 x 60.0 x 2.8 cm^3, about 2 kg per tray) and dried in a Harvest Saver tray dryer (R5-A, Commercial Dehydrator Systems, Eugene, OR, USA) at 45°C and air speed 1 m^3/s until an Aw <0.5. The dried meat was reduced to a coarse powder in a food processor, vacuum packaged and stored at -40°C. Pork loins were purchased on five different days and processed independently to give five true repetitions, each being a mix of at least six loins.

Pemmican: Dried loin (about 160 g) was finely ground in a coffee grinder. Commercial probiotic powder (*Lacticaseilactobacillus rhamnosus* R0011, Lallemand Health Solutions, Montréal, QC, Canada; 0.8 g) was mixed into the ground loin (80 g), followed by filtered beef tallow (50°C; 80 g). Balls (10 g) were formed, individually vacuum packaged and stored in cardboard boxes at RT or 4°C. Pemmican with added cranberry was made following the same process using finely ground loin (65 g), probiotic powder (0.8125 g), dehydrated cranberry bits (16.25 g) and tallow (81.25 g).

Enumeration of bacteria: The method was adapted from Champagne et al. [3,4]. Rehydrating medium (RM; 15 g/l Bacto peptone, 10 g/l tryptone, 5 g/l yeast extract, 2 g/l Tween 80) was brought to 37°C. A pemmican ball (10 g) was added to RM (240 ml) in a sterile 500 ml glass jar and blended (Osterizer blender, Fort Lauderdale, FL, USA) at maximum speed for 30 s, incubated at 37°C for 15 min, and blended for another 30 s. An aliquot (1 ml) was transferred to 1 g/l peptone water (9 ml). Serial 1:9 dilutions were made in 0.1% peptone water and plated in 55 g/l MRS broth with 15 g/l agar using the pour plate method. The plates were incubated at 37°C for 48 h under anaerobic conditions (5% CO_2, 10% H_2, 85% N_2). Probiotic powder (1 g) that had been stored in the same conditions as the pemmican balls was used as a control.

Statistical analyses: Graphs with SEM error bars were prepared with Excel software. Statistics were carried out with SigmaPlot (V15, 2022, Inpixon). Pairwise multiple comparison procedures were made using the Student-Neuman-Keuls system. Microbial CFU data showed variations which could reach >2 log10 in range, therefore, to stabilize variance, all CFU data for each assay were converted to log10 values, and statistical tests were carried out on these log10 conversions.

III. RESULTS AND DISCUSSION

Viable counts of the probiotic cells were followed over an 18-month period (Figure 1). The data of the pemmican with added cranberry are not presented in Figure 1 because values were very similar to those of the basic pemmican formula. Although cranberry can increase the acidity of the product, it was not sufficient to negatively affect viability of the bacteria.

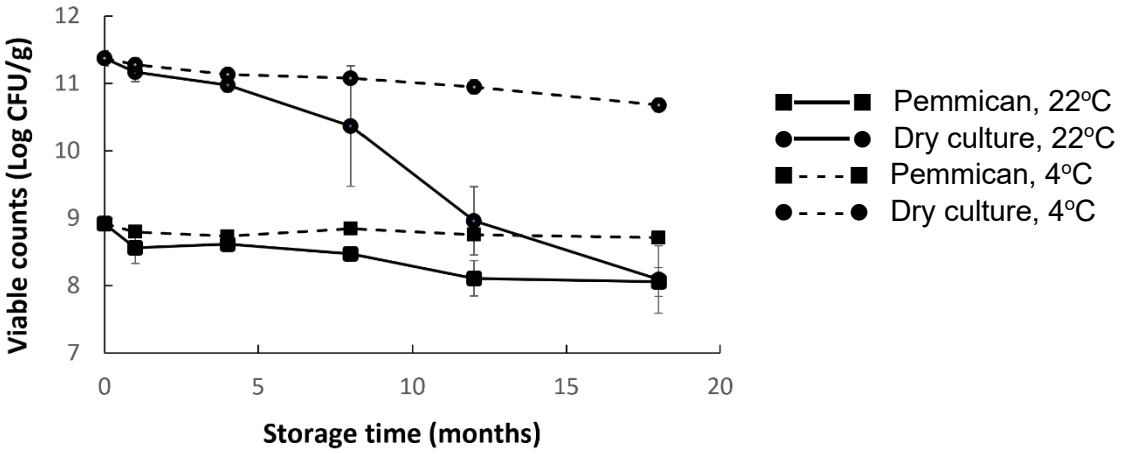

Figure 1: Effect of storage temperature on the viable counts of the probiotic strain *L. rhamnosus* R0011 (for clarity, only select SEM [error bars] are given, representative of variation in the other plots).

In both dried culture and pemmican samples, the losses of viability were significantly greater after 8 months of storage at 22ºC than at 4ºC ($P \leq 0.05$), in agreement with literature [5]. Interestingly, viability losses were higher in the dried culture than the pemmican. This is unusual because adding probiotics to food matrices usually results in strong viability losses [5]. Presumably, this is because the meat was dried and water activity was lower than 0.5 [6]. The high fat content could also contribute to stability, as it can act as a barrier to water and oxygen, particularly when combined with vacuum packaging.

IV. CONCLUSION

The *L. rhamnosus* probiotic used in this study was very stable in a pemmican model. To be recognised as a probiotic-carrying food, products in Canada must contain ≥1 billion cells per portion. For a 100 g portion, the pemmican model studied meets this requirement, even after 18 months storage at 22ºC.

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EFFECTS OF RAPESEED OIL IN SAUSAGES

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I. INTRODUCTION

Fat is an important ingredient in the formulation of sausages which has impact on both sensory and functional properties. Animal fat contains a relatively high proportion of saturated fatty acids which are connected to changes in certain risk markers for cardiovascular disease (i.e. total and LDL cholesterol). Despite controversy on health effects associated with saturated fat [1], exchanging some of the animal fat with plant oils could potentially have a positive health effect for consumers of sausages. Rapeseed oil has a high percentage of unsaturated fatty acids and a mild flavor. However, since plant oils have lower melting point than animal fat an exchange could affect texture and sensory perception of the sausage. The purpose of this study was to compare physical, chemical and sensory properties of grill sausages when saturated fat was partly replaced with rapeseed oil.

II. MATERIALS AND METHODS

A 2x3 factorial design was applied to make grill sausages. All recipes had fixed levels of protein (11%) and fat (18%). Minced meat from pork (23% fat) and beef (14% fat) were used in recipe 1-3, while lean pork (6% fat) and beef (5% fat) were used in recipes 4-6. Pork backfat was the only additional fat-source in recipes 1 and 4, while 50% of the backfat was replaced with rapeseed oil in recipes 2 and 5. All backfat was exchanged with rapeseed oil in recipe 3 and 6. The recipes were equally homogenized and duplicated in randomized order. Alginate (0.2%) was added to all batters to facilitate fat emulsification. The sausages were characterized by several different methods: evaluation by trained sensory assessors, texture profile analysis, fatty acid composition, histology, color measurements, cooking loss and pH. Statistical analyses were performed with the software Minitab (Version 19).

III. RESULTS AND DISCUSSION

The sausages were evaluated for 22 different attributes by the trained sensory panel. For 6 attributes, small but significant differences were found. As shown in Table 1, “recipe 6 sausages” deviated most from the others. These sausages had the highest ($p<0.05$) content of unsaturated fat, still they were found to have higher ($p<0.05$) sensory hardness and lower sensory fatness ($p<0.05$) than the other sausages made with lean meat ingredients. Juiciness was significantly lower ($p<0.05$) for recipe 6 compared to recipes 1-4, but not different from recipe 5. Textural profile analysis (TPA) indicated that springiness and resilience increased when backfat was replaced with rapeseed oil. No difference in cooking loss (approximately 5.5%) was observed between the different recipes. Histological analyses showed that the rapeseed oil was well emulsified in all sausages. Regular meat, with higher animal fat content, gave emulsions with smaller rapeseed droplets than the lean meat ingredients. Color measurements showed increasing lightness L* ($p<0.05$) and higher redness a* ($p<0.05$) when rapeseed oil content was increased. The present results indicate that some of the animal fat could be replaced with rapeseed oil in grill type sausages without detrimental effects on textural and sensory properties. Our findings are in agreement with the results reported by Youssef

and Barbut [2], who compared sausages with different levels of protein. They found high fat loss from batters when protein level was raised to 14%.

Table 1 – Description of sausages and mean values for some of the obtained results. Results with different letters (a-d) are significantly different, p>0.05.

Recipe No.	Description	Ratio sat/unsat fat	Sensory fattiness	Sensory hardness	Sensory springiness	TPA springiness	TPA resilience
1	Regular grill sausage, Control	0.66^{a}	4.76^{a}	3.75^{abc}	3.62^{ab}	65.2^{a}	7.2^{a}
2	Regular meat, 50% of backfat exchanged with rapeseed oil	0.51^{b}	4.68^{a}	3.98^{ab}	3.74^{a}	68.0^{ab}	7.3^{ab}
3	Regular meat, 100% of backfat exchanged with rapeseed oil	0.42^{c}	4.60^{a}	3.89^{ab}	3.79^{a}	70.8^{b}	7.5^{ab}
4	Lean meat and backfat	0.62^{a}	4.84^{a}	3.46^{bc}	2.99^{bc}	69.4^{b}	6.9^{a}
5	Lean meat, 50% of backfat exchanged with rapeseed oil	0.38^{c}	4.41^{a}	3.16^{c}	2.96^{c}	70.5^{b}	7.5^{ab}
6	Lean meat, 100% of backfat exchanged with rapeseed oil	0.17^{d}	3.86^{b}	4.22^{a}	3.88^{a}	75.0^{c}	8.6^{b}

IV. CONCLUSION

This work has shown that exchanging pork backfat with rapeseed oil in grill sausages will improve the ratio of saturated versus unsaturated fatty acids significantly. The results also indicate that fat replacement may not have large effects on sensory or textural properties.

ACKNOWLEDGEMENTS

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SHIITAKE (*Lentinula edodes*) STIPE EXTRACT AS AN ANTIOXIDANT IN FRANKFURTERS WITH SOYBEAN OIL AS ANIMAL FAT SUBSTITUTE

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I. INTRODUCTION

The growing awareness among the population regarding the importance of a healthy diet has increased consumers' concerns about excessive intake of saturated fat, salt, and synthetic additives, driving the demand for more natural and healthier meat products [1]. Consequently, various approaches have been studied to meet consumers' expectations, including substituting animal fat with vegetable oils, such as soybean oil, to improve the lipid profile of meat products [2]. Additionally, the use of natural extracts as substitutes for synthetic additives has gained attention due to consumers' concerns regarding the safety and toxicity of these substances. Shiitake mushrooms and their byproducts, contain phenolic compounds in their composition [3], offering potential as substitutes for commercial antioxidants. This study aimed to evaluate the effect of shiitake stipe extract on the antioxidant activity and oxidative stability of frankfurters with soybean oil as an animal fat substitute.

II. MATERIALS AND METHODS

The shitake stipe extract (SSE) was obtained according to Harada-Padermo et al. [4]. Five formulations of frankfurters were prepared [5]. One was the Control (C) [100% animal fat and 0.05% sodium erythorbate (SE) as an antioxidant], and four were reformulated with 50% of pork backfat replaced by soybean oil: one with SE (0.05%) (S), and three replacing SE with SSE at 0.75% (S0.75), 1% (S1), and 1.5% (S1.5). Pork meat (58%), water (19.64%), sodium chloride (0.75%), sodium tripolyphosphate (0.3%), sodium nitrite (0.012%), and seasoning (0.25%) were used in all formulations. The fatty acid profile (determining the proportion of SFA, MUFA and PUFA) [6], lipid oxidation (TBARS) [6], and antioxidant activity by photochemiluminescence (Photochem, Analytik Jena, TX, USA) were analyzed, with the latter two assessments conducted during 60 days of refrigerated storage. Results were evaluated by ANOVA followed by Tukey's test ($p<0.05$).

III. RESULTS AND DISCUSSION

The incorporation of soybean oil in the formulation of frankfurters significantly ($p<0.05$) reduced SFA and MUFA while increasing PUFA (Figure 1A), demonstrating the positive effect of the partial replacement of pork fat by soybean oil on the lipid profile. Samples containing SE (C and S) showed significantly higher antioxidant activity ($p<0.05$) than S0.75, S1, and S1.5 throughout the storage period (Table 1), indicating that the sodium erythorbate exhibits greater antioxidant capacity than shiitake stipe extract in sausages. Despite this, when evaluating lipid oxidation (Figure 1B), samples S1 and S1.5 did not differ ($p>0.05$) from formulations containing SE (C and S) throughout the storage period. This indicates that SSE, at concentrations starting from 1%, effectively acted as an antioxidant in the frankfurters.

Table 1 - Antioxidant activity by Photochemiluminescence (µg TEAC/g) of frankfurters

Samples	Storage time (days)			
	0	20	40	60
C	0,56±0,01 aA	0,38±0,03 bD	0,49±0,02 aB	0,45±0,01 aC
S	0,60±0,04 aA	0,47±0,01 aB	0,38±0,01 bC	0,35±0,02 bC
S0.75	0,06±0,01 bA	0,06±0,00 cA	0,06±0,00 cA	0,06±0,00 cA
S1	0,07±0,00 bA	0,07±0,00 cA	0,07±0,00 cA	0,07±0,01 cA
S1.5	0,06±0,00 bA	0,06±0,00 cA	0,06±0,00 cA	0,06±0,00 cA

Mean±SD. Different lowercase letters in columns and capital letter in rows indicate significant differences (p<0.05).

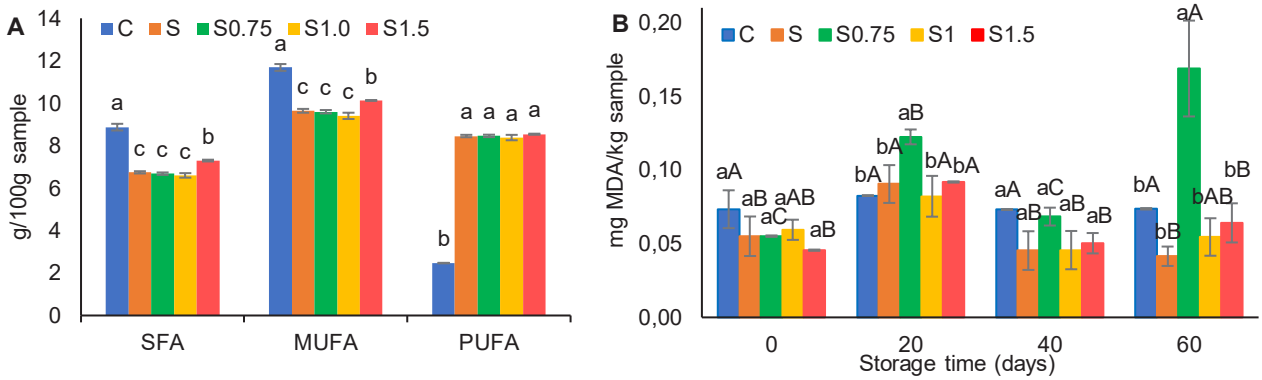

Figure 1. SFA, MUFA and PUFA contents (A) and lipid oxidation (TBARS value) (B) of frankfurters. Different lowercase letters among treatments and capital letters among storage times indicate significant differences (p<0.05).

IV. CONCLUSION

This study demonstrated that substituting pork fat with soybean oil improved the lipid profile of the frankfurters. Sausages with shiitake stipe extract exhibited lower antioxidant capacity than those with sodium erythorbate. However, despite this, shiitake stipe extract, at concentrations starting from 1%, functioned similarly to sodium erythorbate in delaying lipid oxidation of the samples, indicating great potential as a viable strategy for replacing this commercial antioxidant in frankfurters with soybean oil as a substitute for animal fat.

ACKNOWLEDGEMENTS

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Reformulation of dry sausages with natural plant extracts prevents oxidation even in the absence of nitrates

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I. INTRODUCTION

Dry sausages are part of French gastronomy and represent 8% of the total food consumed weekly in France. Appreciated for their sensorial appeal and nutritionally relevant with highly digestible proteins, to name just a few qualities, dry-cured sausages suffer from the presence of food additives like nitrite and nitrate. These additives may lead to harmful nitroso-compounds [1, 2]. Therefore, the replacement of such additives while preserving health and sensorial properties has been the subject of numerous studies, including formulation with polyphenolic compounds. Known for their antioxidant (through scavenging free radicals or chelating metals) and anti-nitrosating properties, polyphenols can be provided by plant byproducts like grapeseed or olive pomace from olive oil extraction [3]. Reformulation provides technological leverage to reduce or even exclude nitrite/nitrate inputs in processed meats. Nitrite/nitrate in dry-fermented pork sausages/salamis can be replaced through formulation with a grapeseed/olive pomace mixture, while controlling microbiological safety [4, 5], albeit with possible color or taste alteration. Preliminary studies have defined an acceptable concentration from a sensory point of view, set at 6 mM eq gallic acid. The aim of the present study was to investigate different sources of polyphenols, alone or in combination with nitrates, to highlight possible synergistic effects on oxidation and prevention of nitrosation.

II. MATERIALS AND METHODS

The sausages were made at the Technical Institute ADIV (Clermont-Ferrand, France), with 87% of porcine shoulder meat and 13% of porcine back fat. Meats were ground at 6 mm diameter and then stuffed in porcine casings of 55 mm diameter. Additions were 160 mg/kg $NaNO_3$ and/or 6 mM polyphenol extracts: green tea GT, olive grape OG, fruit cocktail FC. Products were flavored with 1.50 g/kg of ground grey pepper. Starters (namely *Lactobacillus sakei, Staphylococcus carnosus, Staphylococcus xylosus* (Lallemand, France)), dextrose and lactose (0.15 g/kg; 5.50 g/kg and 6.00 g/kg, respectively) were added to acidify the sausages during the fermentation step. Seven formulations of dry sausage were produced: 0 $NaNO_3$ / 0 $NaNO_2$; 0 $NaNO_3$ / 0 $NaNO_2$ + GT; 0 $NaNO_3$ / 0 $NaNO_2$ + OG; 160 $NaNO_3$ / 0 $NaNO_2$; 160 $NaNO_3$ / 0 $NaNO_2$ + FC; 160 $NaNO_3$ / 0 $NaNO_2$ + GT; 160 $NaNO_3$ / 0 $NaNONO_2$ + OG. The process was similar to that described by [6]. After the fermentation step, the pH increased slowly to final values between 5.1 and 5.2. No significant pH difference was noted for 5 of the 7 trials or for weight loss (44%), except for trials including OG with or without preservatives. For these conditions, pH values after fermentation and drying were higher (+0.1 point) than in other tests.

Determination of thiobarbituric acid reactive substances (TBARS) and nitroso-compounds: The dry sausages were ground in liquid nitrogen to avoid any oxidation and 1 g aliquots were stored at -80°C before use. TBARS were determined as described by [2] and expressed in mg/kg MDA. Nitroso-compounds (nitrosothiols & non-volatile nitrosamines), and residual NO_2 and NO_3, were assessed using the Griess method and expressed in ppm [7]. Nitrosylated heme and total heme were measured as described in [6] and expressed in % and ppm, respectively. Data were analyzed with Jamovi software (version 2.3.26). Variance analysis (ANOVA) and a Tukey *post-hoc* test, as well as PCA were carried out. The significances were given by p values. Every biochemical analysis was performed in triplicate and the results are systematically given as mean ± standard deviation.

III. RESULTS AND DISCUSSION

The results are presented in table 1. In the absence of nitrates, practically no nitrites were detected and the residual nitrates corresponded to the nitrates intrinsically present in muscle. Adding nitrates to the formulation increased both residual nitrites and nitrates. Residual nitrate levels were 50% higher with green tea extracts. Nitroso-compounds, *ie*, RSNO and NNO, were hardly detected, whatever the formulation. The percentage of nitrosyl iron was lower in the absence of nitrates in the formulation,

but with nitrates we observed a decrease of nitrosyl iron when polyphenols were provided in the fruit cocktail. This difference could be due to the nature of polyphenols and their ability to chelate metal. Lastly, the oxidation level of dry sausages was the highest in the absence of nitrates or polyphenols, but was drastically reduced when adding polyphenols. The oxidation level was tantamount to that of the formulation with nitrates alone (160/0). Moreover, in combination with plant extracts, the oxidation level was reduced by almost $^{1}/_{3}$, highlighting a synergistic effect of nitrates and polyphenols.

Formulation NO3/ NO2								
	PP extract	residual NO2 ppm	residual NO3 ppm	RSNO ppm	NNO ppm	Total heme ppm	% Fe-NO	MDA mg/kg
0/0	/	0 +/- 0 a	6.77 +/- 0.18 a	0 +/- 0	0.037 +/- 0. 032	197 +/- 21 a	29.8 +/- 1.4 a	0.133 +/- 0.007 a
0/0 - GT	Green Tea	0.18 +/- 0.03 b	6.18 +/- 0.09 b	0.017 +/- 0.001	0 +/- 0	158 +/- 2 b	31.1 +/- 1 a	0.075 +/- 0.009 b
0/0 - OG	Olive Grape	0 +/- 0 a	6.63 +/- 0.19 a	0 +/- 0	0.057 +/- 0. 050	199 +/- 1 a	27.3 +/- 0.5a	0.078 +/- 0.004 b
160/0	/	2.91 +/- 0.05 e	9.63 +/- 0.06 c	0.065 +/- 0.001	0.096 +/- 0.017	184 +/- 3 a	50.7 +/- 0.5 b	0.076 +/- 0.004 b
160/0 - FC	Fruit Cocktail	2.69 +/- 0.02 d	9.92 +/- 0.15 d	0.047 +/- 0.001	0.025 +/- 0.04	285 +/- 3 c	39.6 +/- 0.5 c	0.056 +/- 0.004 c
160/0 - GT	Green Tea	5.38 +/- 0.08 f	14.2 +/- 0.2 f	0.098 +/- 0.001	0.108 +/- 0.082	313 +/- 25 d	46.6 +/- 0.4 b	0.063 +/- 0.006 c
160/0 - OG	Olive Grape	2.12 +/- 0.02 c	11.3 +/- 0.1 e	0 +/- 0	0.006 +/- 0.01	233 +/- 2 c	56.3 +/- 2.4 b	0.052 +/- 0.001 c
P		p<0.001	p<0.001	p>0.05	p>0.05	p<0.001	p<0.001	p<0.001

Table 1: Mean+/- SD of residual nitrites, residual nitrates, nitroso-compounds (RSNO, NNO, Fe-NO), total heme and lipid oxidation (MDA) in dry sausages formulated with or without nitrates and with or without 6 mM plant extract (green tea GT, olive Grape OG, fruit cocktail FC)

IV. CONCLUSION

Dry sausages did not contain meaningful amount of harmful nonvolatile nitrosamines, which is a public health result worth mentioning. Replacing nitrates in dry sausages is possible with 6 mM plant extract (green tea and olive grape), with limited lipid oxidation. Moreover, the combination with nitrates highlighted a synergistic action on lipid oxidation, therefore ensuring better preservation of the food product. Further investigations are planned to evaluate how the polyphenol extracts protect against oxidation and nitration in digestive conditions, which are known to increase oxidation and the release of NO.

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Formulation with fava bean (Vicia faba L.) or fava bean + flaxseed delayed lipid oxidation in frankfurter and during its digestion.

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I. INTRODUCTION

The sustainability of meat consumption is the subject of much debate, for reasons of ecological footprint. Plant-based ingredients, especially from legumes or seeds can participate to partial replacement of animal protein especially if it adds value [1]. Rich in proteins, fava beans are a good candidate, provided they are obtained using a process that reduces anti-nutritional factors [2]. Moreover, this crop contains vitamin, mineral, dietary fibre, phenols and flavonoids [3]. Flaxseed, an oil-seeds, is well known for its richness in alpha-linolenic acid and is considered as a great source of ω-3 polyunsaturated fatty acids [4]. But, its alphalinolenic acid content is highly susceptible to oxidation [5]. Therefore, we aimed to evaluate the benefice of partial replacement of meat protein by fava bean and flaxseed flour on the nutritional quality of frankfurter.

II. MATERIALS AND METHODS

Frankfurters were manufactured at the laboratory according to [6] with flour inclusion 10 % FB (Fava bean), and 10 % FBFS (75% fava bean + 25% flaxseed), both processed by extrusion and without any flour for control. Textural properties (TPA) protein, lipid content and composition, nitrogen content and oxidation were assessed on frankfurters. The microstructural characteristics were evaluated by light microscopy. Sections of 10 µm thick collected on glass slides were stained for 3 min with red oil for fat droplets and Sirius red for collagen. Micrographs were acquired at 20x magnification using an Olympus BX 61 microscope equipped with a high-resolution digital camera (Olympus DP 71) and an Olympus Cell Sens software (Olympus France SAS, Rungis, France). *In vitro* adult digestion was performed according to [1] in triplicate, bolus were obtained after being masticated to reproduce the granulometry of *in vivo* bolus. Oxidation was determined using TBARS method and expressed in MDA. The total nitrogen content of in digesta was determined by using a micro-Kjeldahl. Moreover, trichloroacetic acid-soluble nitrogen (accounting for all peptides and free amino acids) and phosphotungstic acid-soluble nitrogen (accounting for small peptides and free amino acids) were also determined.
Data were analysed with the software Jamovi (version 2.3.26) [Computer Software]. Variance analysis (ANOVA) and a Tukey *post-hoc* test, as well as PCA were carried out. The significances were given by p values. Every biochemical analysis has been performed in triplicates, and the results are systematically given as mean ± standard deviation.

III. RESULTS AND DISCUSSION

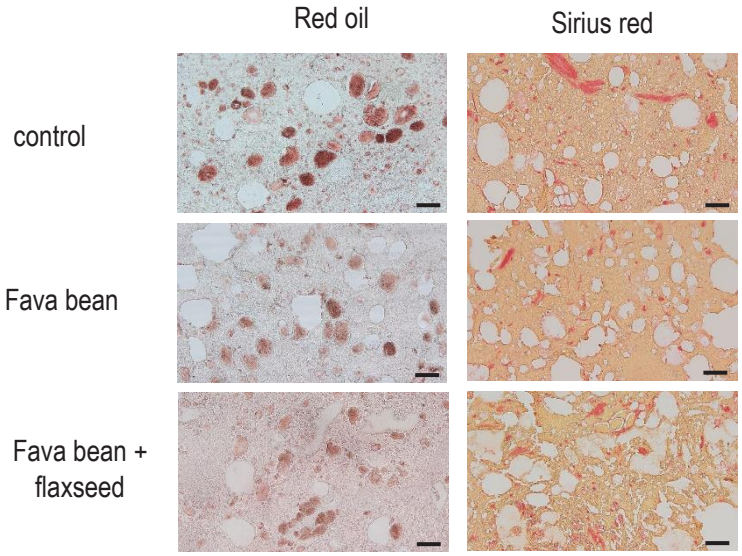
The textural properties differed between frankfurters: firmness, gumminess and adhesiveness were lower with addition of FB and this effect was even more pronounced for FBFS ($p<0.05$). Protein content was higher with FB addition compared to control and FBFS ($p<0.05$). Lipid content was similar (14%) whatever the frankfurter formulation. But during storage, control samples exhibited more lipid oxidation than those formulated with FB or FBFS, this is still the case during digestion. This result was all the more remarkable as polyunsaturated fatty acids were higher for FBFS frankfurter. The microstructure of the frankfurter (figure 1) showed smaller lipid droplets with FB of FBFS, that could explain the softer

texture for FB and FBFS frankfurter. The nutritional quality of the frankfurter is summarized in table 1, values obtained at the end of digestion 240 min, *i.e.* the ileal digesta.

Table 1: nitrogen content from different fraction and oxidation in digesta.

formulation	Nitrogen g/100g digesta	Big peptides g/100g digesta	Small peptides g/100g digesta	Undigested nitrogen g/100g digesta	MDA µM (lipid oxidation)
control	0.351 +/- 0.018	0.171 +/- 0.01	0.095 +/- 0.005 [a]	0.084 +/- 0.007 [a]	34.3 +/- 2.8 [a]
FB	0.376 +/- 0.009	0.166 +/- 0.012	0.115 +/- 0.016 [b]	0.100 +/- 0.002 [b]	24.4 +/- 1.4 [b]
FB FS	0.361 +/- 0.015	0.160 +/- 0.007	0.092 +/- 0.003 [a]	0.102 +/- 0.008 [b]	28.6 +/- 2.7 [c]
P value	NS	NS	0.003	0.022	0.001

A higher quantity of small peptides was found with FB frankfurter, which indicates greater digestibility. In addition, less oxidation was recorded with frankfurters formulated with FB and FBFS.



IV CONCLUSION

Reducing our animal protein intake can be achieved by developing mixed animal and vegetable protein products to reduce our carbon footprint. Moreover, the digestibility of frankfurters was not negatively affected by legume flour, which can be explained by the extrusion process, which reduces or even annihilates anti-nutritional factors. The deficiency in sulphur amino acids in pulses is counterbalanced by those provided by meat.

Figure 1: Histological cross-section of frankfurters using red oil (lipid droplets) and Sirius red (collagen) staining. Bar = 100µm

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Survey of Residual Nitrite and Nitrate in Processed Meats from Small Processors and in Meat Analogues at Retail in the United States

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I. INTRODUCTION

Nitrite (NO_2^-) and Nitrate (NO_3^-) are most common meat curing ingredients that serve as antimicrobials to inhibit *Clostridium botulinum,* limit growth of spoilage organisms, retard lipid peroxidation, and provide the unique cured meat flavor. While the added NO_2^- and NO_3^- concentrations (NO_x^-) in processed meats are strictly regulated worldwide, NO_3^- in processed meats could be inadvertently introduced from various sources including water, non-meat ingredients, processing aids, and meat used. There is a lack of understanding of residual NO_x^- content in different processed meat categories and what factors affect NO_x^- content (Zhang et al., 2023). Therefore, the objectives of the study were to: 1) evaluate all major categories of cured meats for NO_x^- content from small and regional meat processors across three geographic locations in the United States, 2) evaluate the impact of how water source, species of meat, addition of non-meat ingredients and processing methods affect residual NO_x^-, and 3) provide a comparison of the NO_x^- content in plant protein meat analogues available at retail to traditional processed meat products.

II. MATERIALS AND METHODS

Processed meat samples were collected during product competitions hosted by three States' Meat Processor Associations from April 2023 to March 2024. Meat analogues were acquired at retail in Madison, WI in February 2024. Processed meat and meat analogues were homogenized using a polytron blender with phosphate buffer (pH 7.4) followed by methanol extraction prior to being injected into a HPLC analyzer (ENO-20 nitrite and nitrate analyzer with a reverse phase column) for measuring NO_x^-content. Data was collected using Powerchrome 16.0 and statistically analyzed by R studio (R package 4.3.3.). Multi-linear regression was used to assess factors that contributed to residual nitrite and nitrate levels in processed meats. One-way ANOVA test and Pairwise T-test were used to assess statistical differences among each processed meats classification. Correlation analysis (Pearson correlation) was conducted to evaluate the relationship for NO_3^- in potable water and meat samples in Wisconsin.

III. RESULTS AND DISCUSSION

A total of 973 samples of processed meat were collected from Wisconsin (*n*= 462), Pennsylvania (*n*=291), and California (*n*=220) and 53 meat analogue samples were acquired from retail. NO_2^- in processed meat from Wisconsin, Pennsylvania, and California averaged 13.8±1.7, 18.3±2.6, and 10.4±2.3 ppm, respectively. The NO_3^- in processed meat from Wisconsin, Pennsylvania, and California averaged 47.1±2.7, 30.3±2.6, and 8.3±1.0 ppm, respectively. These results were similar to the last survey conducted by González et al. (2012). For plant protein-based meat analogues the average residual NO_2^- was 1.9±0.2 ppm and the average NO_3^- was 7.3 ±0.7 ppm. Multi-linear regression indicated that NO_2^- was affected by geographic location ($P \leqslant 0.001$), fermentation or chemical acidification ($P \leqslant 0.001$), species of meat (pork and poultry, $P \leqslant 0.001$), dehydration ($P \leqslant 0.01$), and inclusion of variety meats ($P \leqslant 0.05$). NO_3^- content (Fig. 1) was affected by geographic location ($P \leqslant 0.001$), species of meat (pork and poultry, $P \leqslant 0.001$), dehydration ($P \leqslant 0.01$), inclusion of variety meats ($P \leqslant 0.05$) and fine or coarse ground ($P \leqslant 0.05$). Correlation analysis for Wisconsin samples indicated potable water usage did not affect the NO_3^- content in the processed meats when correlated ($|r| < 0.25$) with each county's potable NO_3^- levels (Education, 2023).

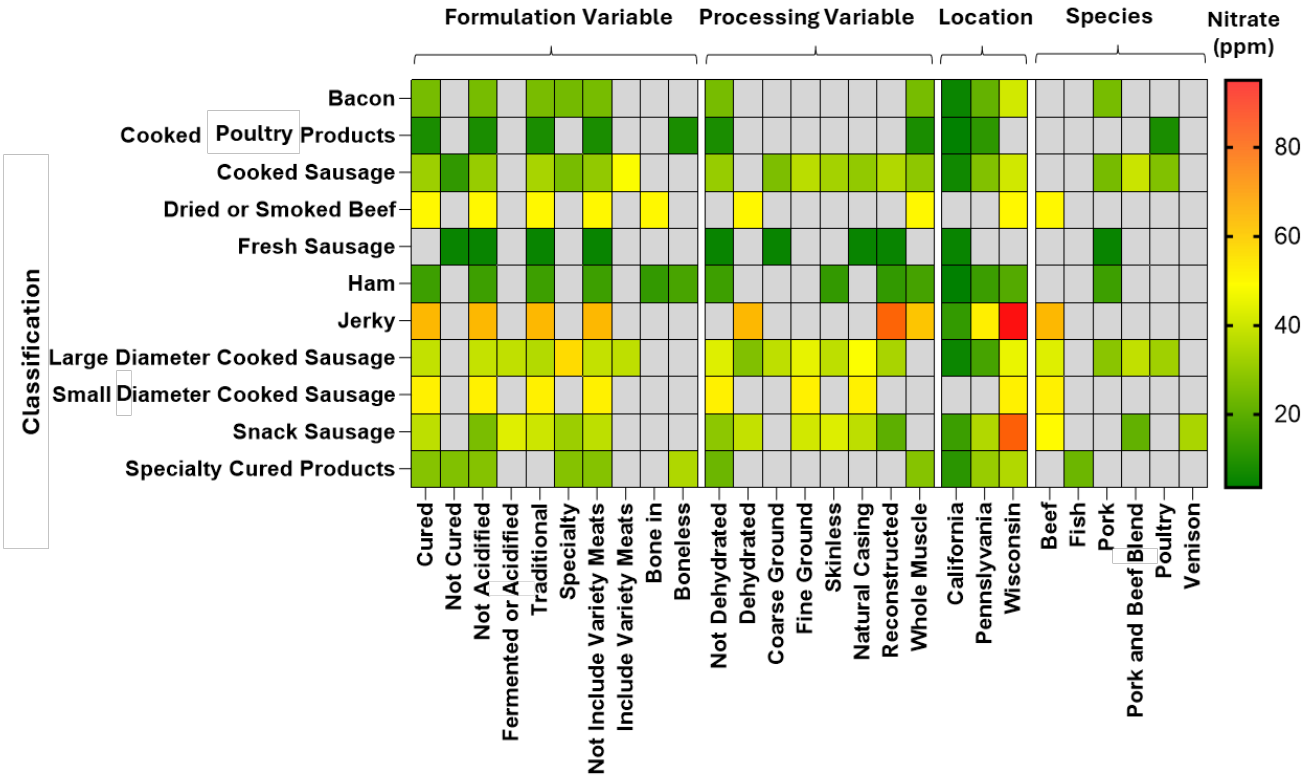

Figure 1. Residual nitrate concentration (ppm) associated with classification and variables in various processed meats.

IV. CONCLUSION

The study presented comprehensive information for consumers and researchers who have interest in residual NO_2^- and NO_3^- as well as evaluated how formulation and processing variables, species of meat, non-meat ingredients, water and geographic location correlated with NO_x^-in all common classification of processed meats. The study suggests that geographic differences of NO_3^- content perhaps was led by differences in spice blend usage in different regions of United States as all other variables (meat, water, other non-meat ingredients) demonstrated a high degree of variability. Moreover, this study provides a baseline that can be used to compare NO_x^- concentration between processed meats and meat analogues.

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Exogenous microRNA kinetics and the importance of animal protein intake in muscle maintenance

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I. INTRODUCTION

Dietary patterns have a profound impact on animal metabolic outcomes and physiological responses. It's well-established that reduced protein intake can disrupt muscle health by increasing muscle protein catabolism [1]. The pathways behind this are intricately linked to the function of macro, micro, and molecular nutrients that epigenetically modulate protein expression at the translation level [2]. MicroRNAs (miRs), small RNA molecules that silence gene expression, are key players in every cellular process leading to cell differentiation, development, and homeostasis [3]. Our previous research has shown that meat-derived miRs can withstand aging, cooking, and digestion and are potentially available for absorption in the duodenum. In this study, we have identified beef-derived miRs not homologous to murine and examined their absorption kinetics and organ distribution in mice. We've also investigated the effects of the absence of animal protein on lean tissue maintenance, presenting novel findings that contribute to our understanding of dietary patterns and muscle health.

II. MATERIALS AND METHODS

Animals and diets: Twelve-weeks-old, C57BL/6J background mice (male, 24.5 ± 0.4) were randomly assigned into two groups comprising four experimental units (n=4). Within each group, the mice were further randomized into two subgroups. One subgroup was fed a BEEF extract (6 µl/g), while the other received a carbohydrate diet (CHO). Mice in both dietary groups were allowed to have a regular chow diet and water ad libitum. Mice were gavaged twice a day within a 12-hour interval for a duration of seven consecutive days. Magnetic Resonance Imaging MRI (EchoMRI^{TM-} 100 System, Echo Medical Systems, Houston, TX) data were acquired from each mouse to establish baseline physiological parameters on day 1 and subsequently on day 7 to evaluate the effect of beef on lean muscle maintenance. On the fifth day of the experiment, all mice were individually housed in Promethion cages (High-Resolution Metabolic and Behavioral Phenotyping Systems for Rodents, Sable Systems International) for 35 hours to evaluate physiological activities. The same experiment was replicated to improve power, totaling 8 experiment units per treatment (n=16). **Sample collection and processing**: Post-euthanasia, organs including the liver, stomach, small and large intestine, muscle, kidney, and cecum samples were collected from each mouse and snap-frozen in liquid nitrogen to preserve tissue integrity until further analysis. **Total RNA extraction, cDNA synthesis and RT-PCR:** To understand the kinetics of beef-derived miRs, total RNA was extracted from each sample type using TRIzol method. MicroRNA expression was quantified using qRT-PCR with the selected primers and 18s as the housekeeping gene. **Statistical analysis:** MicroRNA presence in organs was analyzed as a binomial distribution using a Qui-square test. Resonance Imaging MRI and Promethion metabolic / behavior data were analyzed as a CRD, whereas behavior data was designed as a 2x2 factorial (diet x time). Data were analyzed using SAS.

III. RESULTS AND DISCUSSION

The expression of 10 beef-derived miRs, including bta-miRs 2484, 2340, 2453, and 2440, and miRs 2284w, 2284x, 3432a, 3431, 2422, and 11988 was verified in mice liver, kidney, stomach, large and

small intestines, cecum and skeletal muscle. This study did not find evidence that beef-derived miRs were absorbed in the mice GIT. Beef supplementation did not alter mice behavior and metabolic parameters such as O_2 consumption, CO_2 excretion, kcal/hr, food and water uptake, and physical activity. On the other hand, Mice that received BEEF retained lean mass during a period of 8 days. Mice that did not receive animal protein lost muscle mass (Table 1). Mice fed BEEF also retained more free water than mice fed CHO, possibly due to the maintenance of lean mass.

Table 1. Lean and free water % of mice fed short-term BEEF and CHO diets.

	Day	BEEF[1]	CHO[2]	*P*-value[3]	Std. error[4]
Lean	1	24.78	24.63[A]	0.03	0.30
	8	24.86	24.02[B]		
Free water	1	0.06[B]	0.08	0.01	0.01
	8	0.10[A]	0.06		

[1]Bovine m. semimembranosus extract in DDW.
[2]Starch = 29.81% + Maltodextrin = 9.90% + Sucrose = 11.25% + Soybean oil = 5.28% + 43.76% DDW.
[3]Significant at ≤ 0.05.
[4]Standard error of the mean.

Despite the absence of detectable ruminant-specific miR derived from beef in mice tissues, the observed lean maintenance in beef-fed mice suggests a potential role of dietary protein sources in modulating muscle tissue metabolism. At the end of the experiment, the lack of significant differences in physiological parameters between the carbohydrate and beef-fed groups illustrates the need for long-term dietary treatment for metabolic adaptation and the importance of long-term assessments.

IV CONCLUSION

The absence of the expression of beef-derived miRs in mice organs suggests that those small RNA molecules may not be absorbed in the GIT or the life span of exogenous miRs in the blood, after absorption is short. Based on previous research suggesting that milk and vegetable-derived miRs can be absorbed in in the GIT, it is possible that the short-term approach and post-mortem collection time used in this trial did not allow the detection of miRs in mice organs. On the other hand, the absence of animal protein in diets is detrimental to muscle maintenance. The presence of animal protein in diets is essential to avoid sarcopenia.

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ANTIOXIDANT ACTIVITY OF YERBA MATE (Ilex paraguariensis) EXTRACT IN LOW COST SAUSAGE

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I. INTRODUCTION

The food business is investing a lot of money in ingredient development research as a result of consumer demand for healthier options. The current study evaluated the use of free and microencapsulated yerba mate extract in low cost sausage, emphasizing the preservation of the extract's physical and antioxidant properties during storage at 5 °C. The study's findings demonstrate the potential of yerba mate extract as a useful ingredient for sausages, providing advantages in terms of the food's physical stability, antioxidant capacity, and antibacterial activity during storage.

II. MATERIALS AND METHODS

Low-cost sausage manufacture: 60% mechanically deboned chicken meat, 10.46% pork meat, 10% crushed ice, 2% isolated soy protein, 1.6% salt, 0.25% cured salt (94% salt and 6% sodium nitrate), 0.3% sodium tripolyphosphate, 0.09% sodium erythorbate, 0.8% spices and 2.0 manioc starch made up the formulation of the control low-cost sausage (10 kg each bath).
The three formulations with yerba mate free extract (0.1%), microencapsulated extract (1%) and control without yerbs mate are processed. The sausages were made in accordance with industry standards at Meat Technology Center of the Institute of Food Technology's. The emulsion was stuffed in 22mm celullosic casing and cooked to an internal temperature of 72ºC. Cooking loss was determined (HAYES, 2011).The sausages were kept at 5ºC during storage. The following physical-chemical parameters were asessed at 1 and 45 days of storage: water activity (Aw), chemicals reactive substance to thiobarbituric acid - Tbars (KONIECKO, 1985), objective color (KONICA MINOLTA, 2007), phenolic compounds (ERKAN-KOÇ et al., 2015), and antioxidant activity (JIMÉNEZ-ZAMORA et al.,2016). Data were submitted analysis of variance (ANOVA) and Tukey's Test in order to identify differences ($p<0.05$) between pairs of means with Statistic 7 (Stasoft v.7).

III. RESULTS AND DISCUSSION

The control sample in the low-cost sausage lost 7.72% (0.01) of its weight during cooking; the sample containing free yerba mate extract showed a percentage of 5.39% (0.01), which is a 30% decrease in cooking weight loss compared to the control sample; and the sample containing microencapsulated yerba mate extract showed a percentage of 6.82%.
The free extract sample exhibited a rise in TBARS-reactive chemicals over the course of the days, indicating that the free extract may not have been added in a way that would have prevented lipid oxidation during storage. It is important to assess whether the results were affected by other parameters, such as the extract's stability and the high initial oxidation load of the mechanically deboned poultry meat, or whether the concentration of free extract was sufficient.
It was observed that, in the case of sausages containing microencapsulated extract, microencapsulation might not have offered sufficient defense against lipid oxidation. In low-cost sausages, the inclusion of free or microencapsulated extract did not fully prevent lipid oxidation as compared to the control.

The a* and b* color components increased in control, microencapsulated extract, and free extract samples from day 1 to day 45. (Table 1). Compared to the other samples, the control sample had a higher concentration of phenolic chemicals. Consequently, it is evident how sensitive these compounds are to the usage of a raw material with a high initial oxidation concentration. The free yerba mate extract was shown to be susceptible to the initial oxidation load, as indicated by the antioxidant activity measured by the DPPH and ABTS method (Table 1), however, it was able to maintain or even increase its antioxidant value during the sausage's oxidation process.

Table 1 – Chemical and physical parameters means for low cost sausage containing yerba mate free extract and microencapsulated extract between 1 and 45 days.

Parameters	Dia 01			Dia 45		
	Control	Free extract	Microencapsulated extracted	Control	Free extract	Microencapsulated extracted
Analysis						
Aw[3]	0.966^{a}(0.002)	0.967^{a}(0.000)	0.969^{a}(0.001)	0.960^{b}(0.001)	0.962^{b}(0.002)	0.960^{b}(0.002)
L*[9]	63.52^{a}(0.58)	63.43^{a}(0.70)	64.04^{a}(1.02)	64.11^{a}(0.36)	64.32^{a}(0.54)	65.52^{b}(0.76)
a*[9]	11.81^{a}(0.18)	11.82^{a}(0.45)	10.95^{b}(0.35)	12.30^{a}(0.28)	12.14^{a}(0.30)	11.23^{b}(0.39)
b*[9]	13.96^{a}(0.33)	13.98^{a}(0.36)	14.01^{a}(0.35)	15.08^{b}(0.12)	15.33^{b}(0.26)	15.45^{b}(0.22)
Phenolic compounds (mg EAG/100g)[3]	52.54^{b}(0.55)	48.61^{a}(1.19)	49.53^{bc}(0.14)	51.49^{bc}(0.29)	49.82^{bc}(1.42)	48.61^{c}(1.27)
Antioxidant activity ABTS (µmol TE/g b.u)[3]	3.64^{ab}(0.006)	3.14^{d}(0.013)	3.77^{a}(0.006)	3.49b^{c}(0.010)	3.34^{cd}(0.001)	3.22^{cd}(0.008)
Antioxidant activity DPPH (µmol TE/g b.u)[3]	2.35^{a}(0.004)	1.70^{bc}(0.018)	2.47^{a}(0.008)	1.96^{b}(0.013)	1.79^{bc}(0.016)	1.65^{c}(0.004)
TBARs (mg/kg)[3]	5.53^{a}(0,09)	2.24^{c}(0.02)	3.97^{b}(0.10)	5.04^{a}(0.03)	5.14^{a}(0.03)	5.08^{a}(0.02)

^{abc} Mean values in a row treatment followed by different letter are significantly different ($p<0.05$) from each other
( ) Standard deviation Number of replicates [3]N=3; [9]N=9

IV. CONCLUSION

The use of both free and microencapsulated yerba mate extract showed promise as a natural antioxidant in inexpensive sausages. Notably, using the microencapsulated extract offered additional benefits, particularly for inexpensive sausages. Thus, the findings of this study demonstrate the potential of yerba mate extract as a natural preservative for sausages. Regarding antibacterial and antioxidant properties, yerba mate is a promising ingredient to improve the stability of emulsified sausages.

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NUTRITIONAL AND *IN VITRO* PROTEIN DIGESTIBILITY COMPARISON BETWEEN BEEF, HYBRID, AND PLANT-BASED ANALOGUE BURGERS

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I. INTRODUCTION

Meat alternatives, such as hybrid meat products and plant-based analogues, have been introduced to the market, often leaving consumers uncertain about their nutritional quality, which is directly affected by the ingredients in their formulations. Plant proteins are known to lack some essential amino acids and contain anti-nutrient compounds, potentially affecting their digestibility compared to meat proteins. In this context, this study aimed to compare the nutritional profile and *in vitro* protein digestibility of beef burger (FC), hybrid beef burgers (FH), and plant-based analogue ones (FA and FAF).

II. MATERIALS AND METHODS

Four treatments were elaborated in three batches, as shown in Table 1. The burger samples were prepared by mixing all ingredients in a planetary mixer for 4 minutes. The samples, approximately 100 g each and 12 cm diameter, were immediately frozen at -20°C. The burgers were grilled for 3 minutes on each side at 180°C on an electric grill. Essential amino acids in the burger samples and free amino acids in the digested samples were quantified according to the methodology adapted from previous studies [1]. The in vitro protein digestibility was evaluated according to the Infogest protocol [2] and detailed in a previous study [3]. Differences between treatments were evaluated using one-way ANOVA and the post-hoc Tukey's test with 95% confidence in SPSS software.

Table 1 – Formulations (g/100g) of beef, hybrid, and plant-based analog burgers

Ingredients	Treatments			
	FC	FH	FA	FAF
Lean beef	75	37.5	-	-
Hydrated pea textured protein[1]	-	37.5	75	75
Pork backfat[2]	10	5	-	-
Vegetal fat[3]	-	5	10	10
Methylcellulose	-	-	1.5	0
Flaxseed fiber: psyllium husk (1:1 w/w)	-	-	-	5
Water	13.15	13.15	11.65	8.15

All treatments were elaborated with 1.2% NaCl and 0.6% condiment mix. [1]71.5% moisture, 22% protein, and 1.9% fat. [2] 40% saturated fat. [3]blend of vegetal fat and canola oil (1:1 w/w – 37% saturated fat).

RESULTS AND DISCUSSION

The essential amino acid profiles of the burger samples varied significantly. Analog burgers exhibited the highest levels of calcium, iron, copper, manganese, and magnesium. As expected, the total content of essential amino acids was highest in the beef burger, followed by the hybrid and analogue burgers. The *in vitro* protein digestibility scores also followed this pattern, with the beef burger demonstrating the highest value, followed by FH and FA, and FAF, which showing the lowest protein digestibility, as

shown in Figure 1a. Corroborating these results, the release of free essential amino acids was higher in the beef and hybrid burgers, while the FAF burger showed the lowest value, as illustrated in Figure 1b. Confocal microscopy of the digested samples (Figure 1c) revealed that analogue burgers contained larger protein fragments compared to both beef and hybrid burgers. This suggests that dietary fibres, particularly insoluble ones, may inhibit enzyme activity, thereby reducing protein digestibility. Interestingly, in the FA treatment, a higher number of fat globules was observed (Figure 1c). Methylcellulose, due to its high emulsifying capacity combined with the fact that it is not digested by digestive enzymes probably acted as a barrier to lipid digestibility.

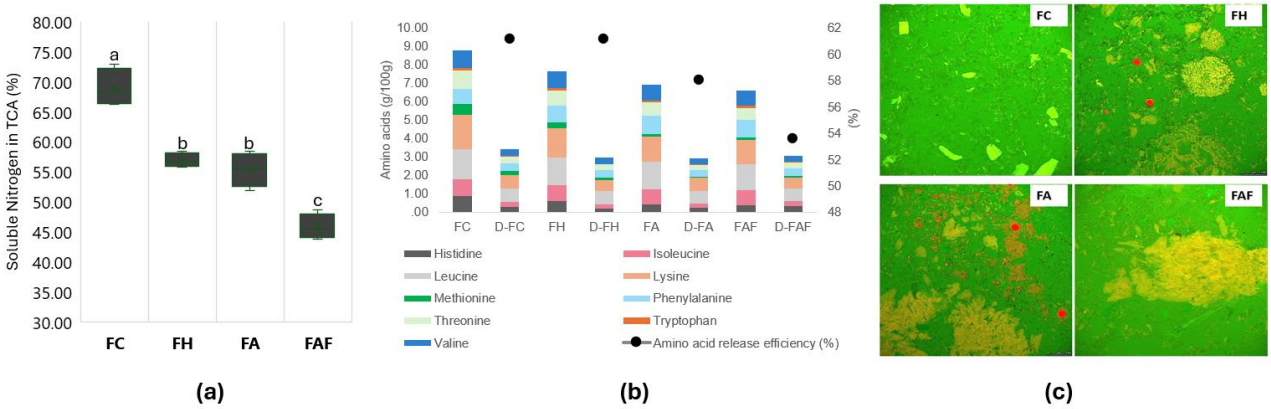

Figure 1. (a) *In vitro* protein digestibility scores based on soluble nitrogen in trichloroacetic acid (10%); (b) Essential amino acids content in the burger samples and in the digested samples (free amino acids) after *in vitro* digestibility protocol and (c) confocal microscopy of digested samples.

III. CONCLUSION

We concluded that pea-based analogue burgers exhibit a good amino acid profile; however, as expected, the level of methionine and the total of essential amino acids was higher in the beef burger. Regarding the *in vitro* protein digestibility, it was higher in the beef burger and lower in the fibre-rich analogue burger. Qualitatively, the analogue burger made with methylcellulose also exhibit reduced lipid digestibility.

ACKNOWLEDGEMENTS

We are grateful to CAPES (Grant No. 001), FAPESP (Grants No. Grant No. 2021/02990-4 and 2019/27354-3), and CNPq (Grant No. 131994/2017-4) for financial support.

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PEPTIDE PROFILING ANALYSIS OF JINHUA HAM BROTH PEPTIDES AT DIFFERENT COOKING TIMES

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I. INTRODUCTION

Dry-cured ham is a premium product with a long history. Its production process can last for 6-24 months [1]. Some of the peptides produced during proteolysis are biologically active and thus potentially beneficial for human health [2]. Numerous reports have demonstrated that Chinese dry-cured ham, including Jinhua and Xuanwei, as well as Spanish dry-cured ham, contain peptides with antioxidative properties [3]. It is also worth noting that the traditional Chinese dry-cured ham has a salt concentration of 6-15%, which is substantially greater than dry-cured ham from European countries [4]. Because of its high salt content, Chinese consumers more commonly use Jinhua ham to prepare broth rather than consume it directly. However, the effects of Jinhua ham broth cooking on the release of peptides have not been reported yet. Therefore, this study aimed to examine the effects of various cooking times on peptide profiles by simulating traditional soup-making conditions.

II. MATERIALS AND METHODS

2.1 Extraction of Jinhua ham broth peptides

The peptides extracted from uncooked ham are referred to as 'uncooked' JHBP-0. Four 'cooked' groups (JHBP-1, JHBP-1.5, JHBP-2, JHBP-2.5) were obtained by cooking with slight modifications based on the method of Zhang et al. [5]. To do so, 200 g of meat were added to 800 mL of water in a ratio of 1:4 (w/v) and boiled at 2100 W using induction cookers, after which the power was adjusted to 300 W to maintain a simmer for 2 h. Water was replenished periodically during cooking to maintain a ratio of 1:4 of meat-to-water, ensuring constant total volume. After filtering, the ham broth was passed through a double-layered gauze to remove insoluble impurities. The ham broth was cooled to room temperature, after which 100 mL of ham broth was mixed with three times its volume of 40% ethanol. After resting the samples at 4℃ for 12 h, centrifugation (12000 g, 10min) followed by rotary evaporation was performed, then freeze-drying at -80℃ in a freeze dryer, and storing of the dried samples at -20℃.

2.2 Characterization of peptide sequences

The desalted peptides were diffused in 0.1% formic acid (solvent A) before being analyzed by Nano LC system coupled with Orbitrap Exploris 480 mass spectrometer with FAIMS (High-Field Asymmetric Waveform Ion Mobility Spectrometry). The analytical columns were applied to perform the chromatographic separation using a 30-minute linear gradient of 3-35% buffer B (80% acetonitrile with 0.1% FA) at a flow rate of 0.3 μL/min. FAIMS had a compensation voltage (CV) of -45 V and -65 V. The mass spectrometer was set to data-dependent analysis (DDA) mode with a dynamic exclusion of 30 s and full-scan MS spectra (m/z 350-1,500) with a resolution of 60,000 (m/z 200) and a resolution of 15,000 (m/z 200) in MS/MS scans.

2.3 Bioinformatics analysis

The peptides extracted from the various broths were identified using PEAKS Studio X Pro (Version 10.6). The UniProt database was used to identify peptides and proteins of origin, with a parent mass error tolerance of 10 ppm and a fragment mass error tolerance of 0.2 Da. Proteomes from *Sus scrofa* (pig) and 'no enzymes' were set as options. For peptide sequences, the processed data used database searching with an FDR≦1%.

III. RESULTS AND DISCUSSION

There were 1,306, 1,352, 1,431, 1,500, and 1,556 peptide sequences found for JHBP-0, JHBP-1, JHBP-1.5, JHBP-2, and JHBP-2.5, respectively (Figure. 1A). Fifty of these peptides were repeated in the five groups, while the unique peptide sequences were 873, 411, 523, 567, and 745 for each group, respectively. Hence, there were 13 identical source proteins in the five groups of which 6, 10, 13, 16, and 20 source proteins being unique to the respective five groups (Figure. 1B). The number of distinctive source proteins was dependent on the cooking time process. In identical source proteins, myosin and actin were the most prevalent sources of peptides (Figure. 1C). The number of peptides from the four source proteins, including myosin, actin, mLC1f and aldolase-fructose-diphosphate A, accounted for more than 50% in each group.

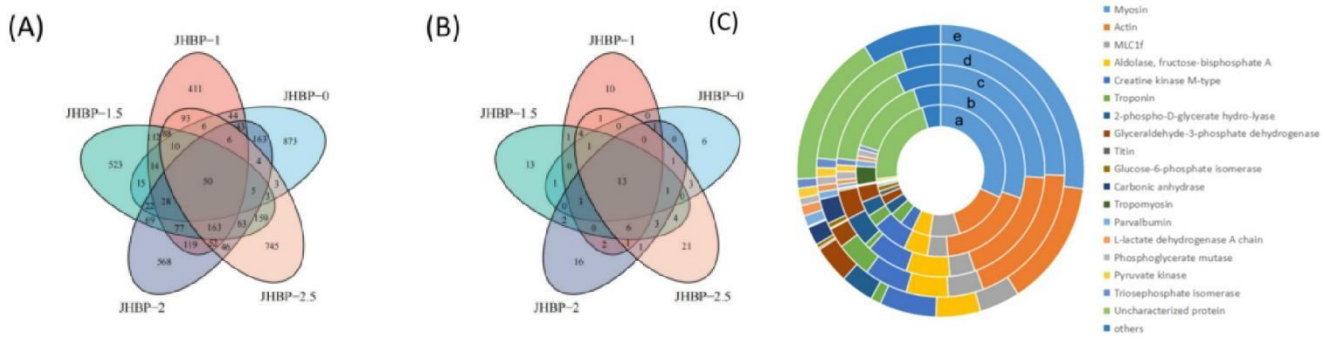

Figure 1. Venn diagram of characteristic peptides (A). Source proteins for peptides (B). Distribution in percentages of peptides according to the origin proteins in muscle of *Sus scrofa*: a. JHBP-0; b. JHBP-1; c. JHBP-1.5; d. JHBP-2; e. JHBP-2.5 (C).

IV. CONCLUSION

The current research revealed that different cooking times altered the peptide profile of Jinhua ham broth peptides. Cooking increased the proportion of <1 kDa peptides and significantly reduced the proportion of peptides in all molecular weight ranges. Meanwhile, cooking increased the abundance of peptides released from myosin and actin, thereby further demonstrating that cooking promotes proteolysis.

ACKNOWLEDGEMENTS

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DEVELOPMENT OF HEALTHY MEAT PRODUCTS: A BIBLIOMETRIC AND SENSORY PERSPECTIVE

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I. INTRODUCTION

Chronic Non-Communicable Diseases (NCDs) constitute one of the main health problems worldwide causing 81% of deaths in Latin America and the Caribbean (1). The excessive intake of sodium and fats has generated this type of problems, for this reason different efforts have been made with the aim of reducing sodium intake, which according to the WHO should be less than 5g per day per person (2), as well as reducing the amount of fat. In this sense, different technological efforts have been developed all over the world, such as the reduction of sodium content in meat products (3,4), fat substitution (5,6). According to ISO 6658, sensory analysis is considered “the tastiest of all sciences” (7). Despite extensive research on meat product reformulation, there is a gap in understanding the effectiveness and consumer acceptance of these strategies. This review aims to explore healthy strategies for meat products, focusing on sensory evaluation and consumer acceptance. It hypothesizes that reformulated products with reduced sodium and fat will meet health guidelines and maintain high sensory acceptance among consumers.

II. MATERIALS AND METHODS

In June 2024, the Scopus database was searched for articles published between 2018 and 2024 with the terms "meat" AND "healthy" AND "strategies" AND "sensory," resulting in 76 documents. Using Rayyan software, authors selected 59 articles focusing on healthy meat product strategies with sensory evaluations, human studies, and within the 2018-2024 timeframe. After a thorough review, excluding non-relevant studies, 38 articles were included, using the PRISMA method (8). The articles were analyzed using the Bibliometrix R package (9) and VOSviewer software (10).

III. RESULTS AND DISCUSSION

The top three articles by citations focus on healthier meat products through fat and sodium reduction (11,12,13). According to the findings, the three countries with the highest number of publications on the subject are Brazil, Spain and Portugal. The analysis of the 38 articles from 2018 to 2024, it is observed that research has focused on developing meat products with healthier profiles. In addition, there is a strong emphasis on developing meat products with fat reductions or substitutions. Reflecting the adaptation of the scientific community to the needs and trends of the population in search of healthier foods taking into account the sensory quality of the product. Co-occurrence analysis identified four clusters: texture and sensory acceptability, animal production, gelling and emulsification, and fat reduction strategies taking into account substitute oils and acid grades for the development of meat products (6). All these clusters demonstrate the possibility of developing healthier meat products.

Of the 38 articles reviewed, there is a distribution of different types of meat products where healthier strategies were applied, where 34% were hamburgers, 32% sausages, 8% salami, 5% pates and 21% other products such as: chorizo, mortadella, etc. Likewise, the strategies applied in the formulation of these products were: 76% made reductions and substitutions of fats in meat products, 11% of the studies made salt reductions, 8% salt and fat combinations, and 5% phosphate reductions, resulting in healthier products. In addition, 14 of them applied descriptive methods to generate attributes for the different types of meat products, and all 38 articles applied affective methods to measure the degree of acceptance of the products, of which 22 studies used the nine-point hedonic scale. This scale has

been widely used since its creation by (14) and remains the most widely used scale. Ten studies used the seven-point hedonic scale, four used the ten-point hedonic scale and two used the five-point hedonic scale. In addition, 87% of the studies had a good acceptance of reformulated meat products and 13% accepted options with the lowest concentration of the substituted product.

IV. CONCLUSION

The analysis of 38 articles reveals a trend toward producing healthier meat products by reducing fats, salt, and phosphates. Most studies focus on hamburgers and sausages, improving texture and nutritional value while maintaining sensory acceptability. Bibliometric tools highlight emerging topics and trends. Affective methods, particularly the nine-point hedonic scale, are widely used to measure consumer acceptance. Overall, reducing fat and sodium are viable strategies for healthier meat products

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SESSION 13

Consumer Topics

Friday 23 August 2024

IMPACT OF ENVIRONMENTALLY FRIENDLY PRACTICES AND ANIMAL MANAGEMENT ON THE SENSORY PROFILE OF PREMIUM POULARD MEAT

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I. INTRODUCTION

The poulard, which is a castrated female chicken, can be considered a premium option in the poultry market. Its meat tends to be juicier and with a more pronounced taste than that of conventional chicken, making it appreciated by discerning consumers and in the realm of gourmet gastronomy [1]. However, these benefits may clash with current population ideals, as consumers not only seek products with excellent sensory quality but also care about animal welfare and its relationship with the environment [2]. Therefore, the objective of this study was to research the sensory profile of poulard meat raised under a semi-extensive regimen (more considerate with animal welfare), comparing a diet based on commercial feed with diets more environmentally friendly that included by-products or healthy seeds.

II. MATERIALS AND METHODS

To achieve these objectives, after being raised on a starter diet for 3 months, 40 poulards were randomly divided into 4 groups of 10 animals per group for different feeding types. The control feed (CO) consisted solely of corn, wheat, and peas. The other feeds included 5% beer bagasse (BB), 5% olive pomace (OP), or 5% flax seed (FS) in their formulation. The fattening period extended for an additional 3 months and was conducted under semi-free-range conditions. After 24 h post-slaughter, breast samples were extracted for sensory analysis. Cooking was carried out in a convection oven at 200 °C until the meat reached an internal temperature of 80 ± 1 °C. The sensory analysis adhered to the UNE-EN ISO 8589:2010 standard and engaged a panel comprising 20 participants. Ten participants were designated for quantitative descriptive analysis (QDA), while the remaining ten conducted an acceptability and preference test. QDA involved a structured scale ranging from 1 to 10 to assess odor, hardness, juiciness, and taste. Additionally, acceptability was evaluated using a 7-point hedonic scale, alongside preference assessments. The collected data underwent evaluation using a 2-way Mixed Model ANOVA, where both feed and panelists served as independent variables, for QDA evaluation and acceptability, while the evaluation of preference was performed using the Friedman test with XLSTAT (Addinsoft, NY, USA). Leas Square Means were separated using Duncan's *post hoc* test (significance level $P < 0.05$).

III. RESULTS AND DISCUSSION

Given that QDA is a detailed and reliable sensory method used to thoroughly evaluate a product across all its sensory attributes, the results obtained in this study (Figure 1) demonstrated that the inclusion of by-products such as BB or OP, as well as FS seed, did not have a significant effect ($P > 0.05$) on the sensory profile of high-quality birds like poulards. These results were similar to those obtained by Krawczyk et al. [1] who observed almost no influence after modifying the diet in poulards of different ages. This could be due to the relatively low inclusion (5%) of BB, OP, and FS, moderating their effect on the sensory parameters. Similarly to the findings in the QDA, the acceptance test revealed no significant differences among the poulard breast samples from those fed the CO diet and those from

the rest of the diets (i.e., BB, OP, and FS) (Figure 2A). This aligns with the notion that the inclusion of 5% of these by-products and this seed does not impact the sensory quality of the meat.

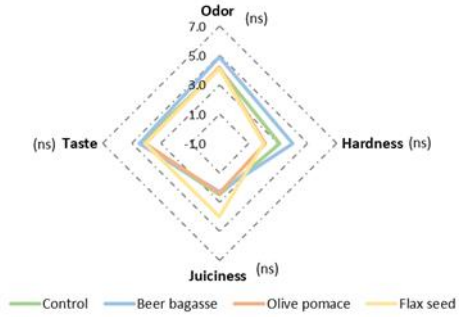

Figure 1. Effect of the diet on the sensory profile of poulard breast raised in semi-free-range conditions (ns: no significant difference, P > 0.05 (Duncan's test)).

Regarding the preference test (Figure 2B), FS poulards were selected as the preferred samples in 38% of cases, followed by OP poulards (37%). On the other hand, the BB and CC poulards obtained lower preferences (13 and 12%, respectively). However, the differences among the four diets were not significant ($P > 0.05$), once again highlighting the potential of these ingredients in poulard feeding.

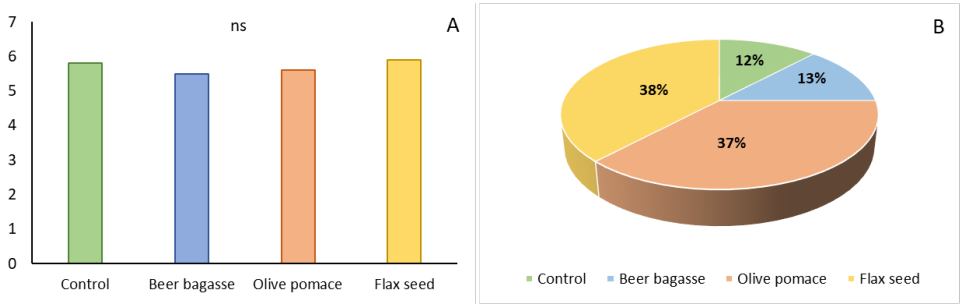

Figure 2. Effect of the diet on the acceptance test (A) and preference test (B) of poulard breast raised in semi-free-range conditions (ns: no significant difference, P > 0.05 (Duncan's test)).

IV. CONCLUSION

The obtained results suggest that the inclusion of BB, OP, and FS in the feeding of semi-free-range poulards did not significantly affect the sensory quality of the meat. These findings supported the potential of using these alternative ingredients in the poultry industry, offering high-quality products without compromising animal welfare or the environment, thus meeting current consumer demands.

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Consumer expectations for beef in the French region Auvergne-Rhône-Alpes

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I. INTRODUCTION

In France, beef consumption has been in steady decline for many years [1], from 25.8 to 22.3 kg/capita/year between 2010 and 2023 (-1.1% per year) [2], although it remains the third most consumed meat in France after pork and chicken [2]. The reasons are multifactorial [3] and linked to (**i**) the controversial image of beef (its environmental impact, the competition between feed and food, respect for animal welfare), (**ii**) the change in consumption patterns (reduction in the daily time devoted to cooking and eating), (**iii**) its high price and, finally, (**iv**) its variable sensory quality [4]. Thus, consumers are not always satisfied with the organoleptic quality of beef [5]. In this context, the scientific project OABov-AURA (2022-2024) was carried out to better identify consumer expectations in the French region of Auvergne-Rhône-Alpes (AURA). This region is the second largest in terms of beef cattle in France (after Nouvelle-Aquitaine), with a strong consumer base offering marketing opportunities for regional production. Therefore, **the aim of this study to better identify consumer expectations** regarding the intrinsic and extrinsic qualities of beef.

II. MATERIALS AND METHODS

The online survey was formatted using LimeSurvey. Before distributing the survey, project partners pre-tested the questionnaire to identify any errors or incomplete responses. The survey was then disseminated to AURA residents online at various periods between April 15, 2022, and December 11, 2023. The responses were analyzed using R software.

III. RESULTS AND DISCUSSION

This study involved 712 respondents, mostly female (62%), young (50% < 37 years of age *vs* 25% > 52 years of age), students (25%) and executives (33%), with a good knowledge of farming (51%), and preferring to buy beef in butcher shops (27%).
Of those surveyed, 48% reported having reduced their meat consumption in recent years (compared to 42% who reported no change). The two main reasons for the decline in beef consumption are explained by health (52% of responses) and ethics (44%) issues.
The majority of consumers (51%) said that eating beef for pleasure was their primary motivation, and are not disappointed with raw (83%) or cooked (54.5%) beef.
Origin (44%), proximity (38%) and the presence of signs of quality and origin (SIQO) (34%) are the most important extrinsic criteria (% of respondents who selected the answer: “very important”). However, taste (62%), tenderness (59%) and freshness (58%) are the most important intrinsic criteria of beef for the respondents (Figure 1).
Consumers also prefer meat that is bright red (72%) and rather fatty (64%).

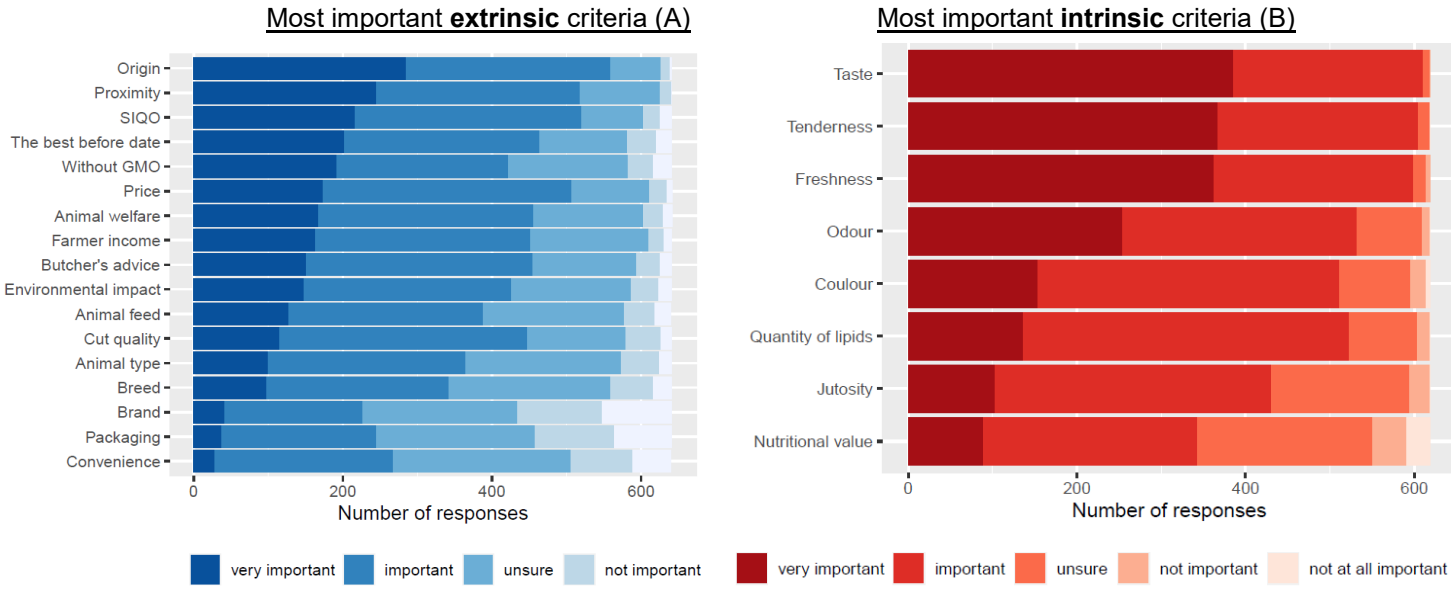

Figure 1. Most important intrinsic (A) and extrinsic (B) criteria of beef quality

Our results align with those of Liu et al. [1] on the extrinsic criteria of beef quality. They found that the most important factors when purchasing food products are sensory quality (67%), price (56%), food safety (47%), origin/traceability (45%), ethics (42%), nutritional value (35%), and environmental impact (33%). Respondents of our study considered taste (flavour) the most important intrinsic criterion for beef, which is consistent with the findings of Liu et al. [6] on a European scale.

IV. CONCLUSION

In conclusion, it is important that consumers in the AURA region have access to various meat types in butcher shops, mainly of French origin and under quality signs to better meet their diverse expectations.

ACKNOWLEDGEMENTS

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EVALUATION OF THE UMAMI INTENSITY OF BEEF HYDROLYSATE WITH DIFFERENT PROTEASES THROUGH CHEMICAL, ELECTRONIC TONGUE, AND SENSORY APPROACH

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I. INTRODUCTION

The demand for healthy and flavorful foods has been increasing among consumers. Umami, the fifth taste, is closely related to protein-rich foods that are highly nutritious [1]. Umami peptides, a group of peptides that enhance the umami taste, have advantages in terms of nutrition, safety, and biological functions [2]. As a result, many efforts are being made to explore umami peptides from foods. These peptides can be obtained through enzymatic hydrolysis; however, their effectiveness varies depending on the protease type and hydrolysis condition [3]. While previous studies have explored the optimal hydrolysis condition for various foods, nonetheless, less attention has been given to the hydrolysis condition of beef products. Therefore, we compared the umami intensity of beef hydrolysates from five protease treatments using chemical evaluation, electronic tongue analysis, and descriptive analysis to determine the optimal hydrolysis condition for the investigation of novel umami peptides from beef.

II. MATERIALS AND METHODS

Beef m. semimembranosus was obtained from three 34-mon steers. Alcalase® 2.4 L from *Bacillus licheniformis* (AL), Flavourzyme® from *Aspergillus oryzae* (FL), and Protamex® from *Bacillus* sp. (PR) were supplied by Novozymes (Bagsvaerd, Denmark). Papain T100 MG (PA) was purchased from Djzymes (Seoul, Korea). Trypsin from porcine pancreas (TR) was obtained from Sigma-Aldrich (Yongin, Korea). Beef samples (8 g) were added into 32 mL of deionized water and homogenized. The temperature and pH were adjusted according to manufacturers' instruction. Proteases were added to the mixture (2% of beef sample, w/w) and incubated for 4 h (AL, FL and PR) or 6 h (PA and TR) based on the preliminary results for the degree of hydrolysis. The samples were neutralized, heated at 95°C for 10 min to inactivate the enzymes, and lyophilized. The umami intensity was evaluated by (1) equivalent umami concentration (EUC) using umami-related amino acids (aspartic and glutamic acid) and 5′-nucleotides (5′-AMP, 5′-GMP, and 5′-IMP) [1], (2) electronic tongue [4], and (3) descriptive analysis. Eight sensory panelists were recruited, trained for the analysis, and evaluated the intensity of sweet, salty, sour, bitter, and umami tastes of the samples (50 mg/mL) using a 10-point scale, which was approved by the Institutional Review Board of Seoul National University (IRB No. 2306/001-003).

III. RESULTS AND DISCUSSION

Recent findings showed that aspartic and glutamic acids, 5′-nucleotides, and umami peptides largely contributed to umami taste [1-3]. Here, FL treatment led to the highest content of aspartic and glutamic acids in beef hydrolysates (Table 1). FL has both exo- and endo-peptidases and can degrade the protein into small peptides and free amino acids easily [3]. On the other hand, AL-treated hydrolysates showed the most abundant nucleotide contents. As a result, AL group had the highest EUC value, followed by FL. However, EUC has limitation that it considers only the synergistic effect between amino acids and nucleotides. On the other hand, both electronic tongue and descriptive analyses showed that AL-treated hydrolysates had the lowest umami intensity and strong bitter taste (Figure 1). In contrast, FL-treated hydrolysates were evaluated as umami-rich samples. Furthermore, the panelists rated high scores for the salty and sweet taste and low scores for the bitter taste of FL-treated

hydrolysates. Umami peptide was reported to enhance salty and sweet taste through synergistic effect and suppress bitter taste [2]. PR and TR groups showed unwelcomed tastes, such as strong bitter and sour tastes, as shown in Figure 1. PA-treated hydrolysates had a weaker umami taste than FL, as judged by panelists.

Table 1 – Umami-related amino acid and nucleotide content and equivalent umami concentration (EUC) of beef hydrolysates with different protease treatments

	AL	FL	PA	PR	TR	SEM
Aspartic acid (g/kg hydrolysate)	3.42^{B}	7.90^{A}	2.24^{D}	1.49^{E}	2.67^{C}	0.081
Glutamic acid (g/kg hydrolysate)	7.21^{B}	17.89^{A}	7.49^{B}	5.02^{C}	6.79^{B}	0.156
5′-AMP (g/kg hydrolysate)	0.20^{A}	0.06^{D}	0.10^{C}	0.07^{D}	0.16^{B}	0.003
5′-IMP (g/kg hydrolysate)	0.46^{A}	0.20^{C}	0.30^{B}	0.02^{E}	0.06^{D}	0.005
5′-GMP (g/kg hydrolysate)	0.10^{A}	0.01^{C}	0.02^{C}	0.04^{B}	0.02^{C}	0.005
EUC (g MSG/100 g)	67.18^{A}	56.42^{B}	34.63^{C}	8.73^{D}	11.15^{D}	1.920

SEM, standard error of mean (n = 15).
$^{A-E}$ Different letters within the same row indicate significant differences ($p < 0.05$).
AL, Alcalase®; FL, Flavourzyme®; PA, papain; PR, Protamex®; TR, trypsin.

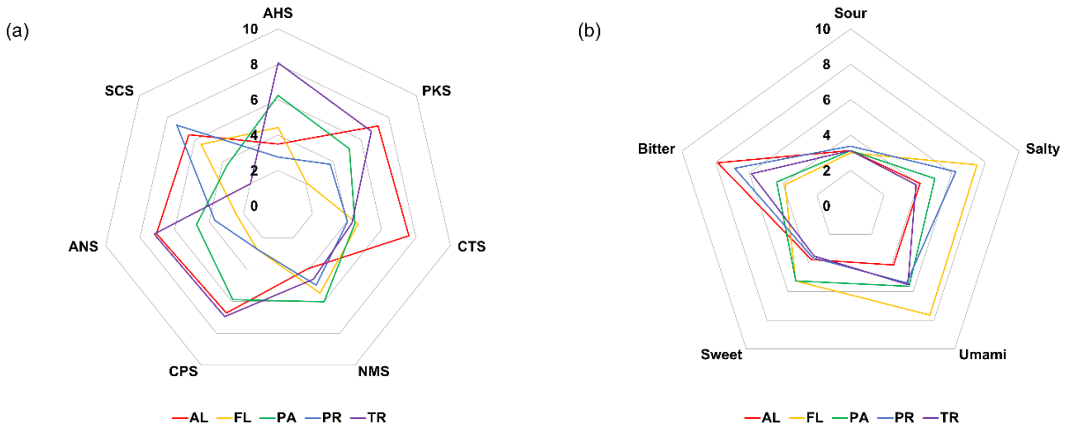

Figure 1. Electronic tongue (a) and descriptive analyses (b) for beef hydrolysates with different protease treatments. The sensors AHS, CTS, NMS, ANS, and SCS respond to sour, salty, umami, sweet, and bitterness, respectively, while PKS and CPS represent universal taste intensity. AL, Alcalase®; FL, Flavourzyme®; PA, papain; PR, Protamex®; TR, trypsin.

IV. CONCLUSION

Beef hydrolysate with Flavourzyme® showed an overall strong umami intensity among hydrolysates in this study. It would be a useful material for exploring novel umami peptides derived from beef.

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NEOPHOBIA NEGATIVELY IMPACT THE PREDISPOSITION TO CONSUME INSECTS

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I. INTRODUCTION

Currently, to solve the problem of lack of protein sources, new foods are being introduced into the market, including edible insects, which show promise in nourishing the growing world population without compromising sustainability [1]. However, there are challenges that need to be overcome to introduce insects as a viable food option, which includes safety, techno-functional properties and consumers perspective [2]. The pretense of humans feeding out of insects relies on the acceptance of the population as a whole, through availability and familiarity to the product [3]. The majority of people view, because of aesthetic and psychological reasons, insects as harmful, dirty animals, pests [4]. However, Brazil is acknowledged worldwide for its “hotspot” status of biodiversity [5] and transfigures as a promising country to entomophagy [6]. The goal of this study is to investigate Brazilian consumer’s perception in relation to neophobia associated with entomophagy. The study released in this short paper is the initial part of a project, which investigates the predisposition of individuals on eating hybrid meat products with addition of insect flour, in order to make consumption of insects acceptable/acquainted.

II. MATERIALS AND METHODS

This study applied two methodologies: food neophobia scale [7] was used to collect data in a 5-point Likert scale format to precisely investigate food neophobia; and, projective technique, was applied using the sentence completion test [8], in which participants finish a sentence instinctively justifying their opinion, in this case, about entomophagy. The Google.forms research management program was utilized to collect data, resulting in 469 responses. Using the software SPSS v.28 the outcomes of the two methods were associated using a chi-square distribution. In the application of statistics, the extreme points of the 5-point Likert scale were grouped, resulting in a 3-point scale (1- agree, 2- indifferent, 3- disagree). The qualitative data from the projective technique were grouped into three different quantitative levels of predisposition to entomophagy: 1- interested, 2- indifferent, 3- reluctant. This study was approved with the following CAAE number 65016922.0.0000.5422 by the FZEA/USP ethics committee.

III. RESULTS AND DISCUSSION

The association between the degree of agreement among Brazilian research subjects about neophobia and the degree of entomophagy predisposition is shown in Table 1. All results are statistically significant at the 0.001 level. In summary, our results corroborate previous studies [2,4] which indicates that an individual's inclination to consume insects decreases as their level of food neophobia increases. Specifically, in the first statement (Table 1), 70.7% of individuals who demonstrated an interesting predisposition to consuming insects agree “to constantly try new and different foods”, on the other hand, only 33.6% of individuals reluctant to consume insects agree with of this statement. Regarding the second statement, which reads, "If I don't know what a food contains, I won't try it," 33.5% of those surveyed said they would be interested in consuming insects, while 61.6% of those who agreed with the statement said they would not. Lastly, respondents who say they eat practically everything (71.7%) are also interested to eat insects, while only 36% of individuals reluctant to entomophagy agree that they eat practically everything. These findings indicate that, in order to

ensure that the population has access to this source of protein in the future, measures that reduce the aversion to insects must be investigated. Studies [1, 3] recommend employing masked insects when preparing food and/or associating them with familiar flavors to reduce insect neophobia.

Table 1 – Association between neophobia and entomophagy predisposition in %.

Statement about neophobia	Likert Scale	Predisposition to entomophagy			P
		interested	indifferent	reluctant	
I am constantly trying new and different foods	agree	70.7a	55.9b	33.6c	<0.001
	indifferent	13.1b	12.4b	16.8a	
	disagree	16.2c	32.0b	49.6a	
If I don't know what a food contains, I don't try it	agree	33.5c	45.1b	61.6a	<0.001
	indifferent	17.3a	16.3a	10.4b	
	disagree	49.2a	38.6b	28.0c	
I eat practically everything	agree	71.7a	60.8b	36.0c	<0.001
	indifferent	7.9b	11.1a	5.6b	
	disagree	20.4c	28.1b	58.4a	

Average values in the same row with different letters indicate significant difference ($p < 0,001$). (n=469).

IV. CONCLUSION

Understanding that there is a large percentage of Brazilian individuals who have food neophobia towards insects, we suggest that these be incorporated into hybrid meat products for better consumer acceptance, since the way insects are inserted (flour) in, for example, hamburgers and sausages, make their appearance, texture and flavor attenuated.

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EFFECT OF FRESH DATE *BAGASSES* ON THE PHYSICOCHEMICAL AND SENSORY PROPERTIES OF BEEF BURGER

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I. INTRODUCTION

The trend towards healthy and sustainable food encourages the design of meat products that benefit both human health and the environment. In meat products, to achieve these objectives, the agrifood coproducts addition is one of the more successfully strategy. Thus, these types of foods are enriched with several nutrients and health benefits. Furthermore, this approach aligns with sustainable production practices and the efficient use of co-products, therefore favoring an efficient use of natural resources and supporting a sustainable food system within the framework of the circular economy.

Dates are noted for their high nutritional profile, rich in dietary fiber, minerals (K, Ca, Mn, etc.) unsaturated fatty acids (oleic and linoleic acids), micronutrients (riboflavin, niacin, tocopherols), and bioactive phytochemicals (phenolic acids, polyphenols and carotenoids). These bioactive components significantly improve their functional properties, antioxidant activity, and healthy benefits [1]. Non-commercial dates have the potential to emerge as natural, functional, and sustainable food ingredient, possessing significant nutritional and bioactive attributes.

The aim of the study was to evaluate the addition of fresh date bagasse at different concentrations (4%, 5% and 6%) as a natural sustainable ingredient to improve the physicochemical and sensory properties of beef burgers.

II. MATERIALS AND METHODS

Preparation of natural ingredient from non-commercial date bagasse. Date bunches were harvest and the non-commercial dates were selected, peeled and pitted. The sugar content of the bagasse was reduced by water extraction until bagasse reach ≤8º Brix. pH and color coordinates were determinate.

Burger's elaboration process. Four independent batches of beef burgers were prepared with different concentration of date bagasse: 0%, 4%, 5% and 6%. All burgers had the following formulation: beef meat (100%), salt (1.5%), parsley (0.5%) and garlic powder (0.05%). After meat grinding, meat and the other ingredients were mixed for 3 min, and then, separated into 4 batches to the incorporation of the different percentages of date bagasse. Finally, they were shaped into pieces (90g).

The physicochemical properties of each batch were analyzed: pH (GLP 21 pH meter), CIEL*a*b* color coordinates (CM-700d spectrophotometer) and texture profile (TA-XT2i Texture Analyzer). For texture analysis, cooked burger pieces (2 cm × 2 cm × 2 cm) were submitted to two-cycle compression to 75% and a constant velocity of 1 mm/s. The parameters determined were hardness (kg), springiness, cohesiveness, and chewiness (kg×cm). Sensory analysis was carried out with an untrained panel (n=20). A seven-point hedonic scale was used, and the panelists were asked to score the cooked beef burgers from 1 (dislike extremely) to 7 (like extremely) on six attributes: visual aspect, global color, global flavor, hardness, taste and general acceptability. Statistical analyses were carried out using the statistical package SPSS v. 24 for Windows (SPSS INC., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

The natural ingredient from non-commercial date bagasse presented a pH of 7.48±0.23, L* of 35.6±1.23, a* of 4.63±0.36 and b* of 9.19±1.34. The raw and cooked beef burgers characterization are showed in Table 1. The addition of date bagasse affected L* (raw burger), b* (cooked burger), hardness and springiness *(p*<0.05), and did not affect the rest of parameters under study. The non-meat ingredients can affect meat products color [1,2]. The date addition at 4 and 5 % decreased the L*value while at a concentration of 6% the L* value was similar to raw burgers control values. After cooking, only b* was affected, decreasing with the date bagasse's addition. At the lowest concentration tested, the hardness and elasticity values decreased (*p*<0.05), but no differences were observed at higher concentrations (*p*>0.05).

Table 1.- Physicochemical properties of raw and cooked beef burgers with different fresh date bagasse concentrations (4, 5 and 6%)

		Batches			
		A (control)	B (4% added date)	C (5% added date)	D (6% added date)
Raw burgers	pH	6.08±0.09	6.09±0.08	6.08±0.02	6.08±0.01
	L*	45.58±2.17^{a}	49.06±0.77^{b}	48.93±0.78^{b}	44.01±2.09^{a}
	a*	5.93±1.76	8.71±0.48	7.10±1.24	6.30±1.70
	b*	7.75±1.53	9.33±0.61	8.72±0.65	6.66±1.62
Cooked burgers	L*	42.52±5.24	41.41±1.30	40.76±1.88	42.71±0.77
	a*	3.85±0.75	3.83±0.81	3.18±0.50	4.81±0.29
	b*	7.27±0.53^{b}	4.83±1.34^{a}	4.51±0.50^{a}	5.50±0.56^{a}
	Hardness	4.12±0.87^{b}	2.45±0.54^{a}	4.85±0.73^{b}	3.60±0.94^{ab}
	Springiness	0.42±0.04^{ab}	0.35±0.07^{a}	0.44±0.03^{ab}	0.45±0.10^{b}
	Cohesiveness	0.35±0.09	0.50±0.14	0.34±0.04	0.41±0.06
	Chewiness	6.21±2.59	4.21±1.32	7.07±1.16	6.82±2.72

Date presented as mean ± standard deviation. In each row, values followed by different letter are significantly different according to the Tukey test (p≤0.05): Samples without letter did not show significant differences (p>0.05).

The sensory analysis showed that burgers with added dates were better evaluated, which was proportional to the percentage of dates added. Therefore, 6% was the highest overall scored.

IV. CONCLUSION

The use of fresh date bagasse as food ingredient in meat products is a viable technological strategy for reformulation of more sustainable meat products. The addition of up to 6% did not affect the quality characteristics and improved the sensory evaluation and acceptance of this meat product. The valorization of non-commercial dates and their application as a food ingredient will promote new opportunities in the development of more sustainable and nutritious meat products.

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SENSORY CHARACTERIZATION OF LOINS FROM ENTIRE MALE, EARLY AND LATE IMMUNOCASTRATED PIGS

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I. INTRODUCTION

Surgical castration is widely used in pig production to avoid boar taint, an unpleasant odour and flavour that occurs in the meat of some entire male pigs. It is mainly due to two compounds, androstenone with a urine-like odour and skatole with a faecal-like odour [1]. Immunocastration is an alternative to surgical castration and consists of the administration of at least two doses of a vaccine that immunises against gonadotropin-releasing factor (GnRF) and suppresses testicular activity. The timing of each dose might affect animal performance, carcass and meat quality characteristics [2]. In general, the carcasses of entire male pigs were leaner and had a higher shear force than those of immunocastrated pigs, and their fatty acid composition was also different All this, together with the suppression of the testicular activity, may influence the sensory characteristics of the pork. The aim of the present work is to sensory characterize pork from entire male (EM) and from early (EIC) and late immunocastrated (LIC).

II. MATERIALS AND METHODS

The loins of 37 pigs were obtained near the last rib. Eleven loins were from EM pigs, 12 from EIC pigs and 14 from LIC pigs. Immunocastration was performed with Improvac® (Zoetis, Madrid, Spain) in two doses. In EIC, the first dose (V1) was administered 13 weeks before slaughter and the second dose (V2) 5 weeks later, i.e. 8 weeks before slaughter. In LIC, V1 was administered 8 weeks before slaughter and V2 4 weeks later, i.e. 4 weeks before slaughter.

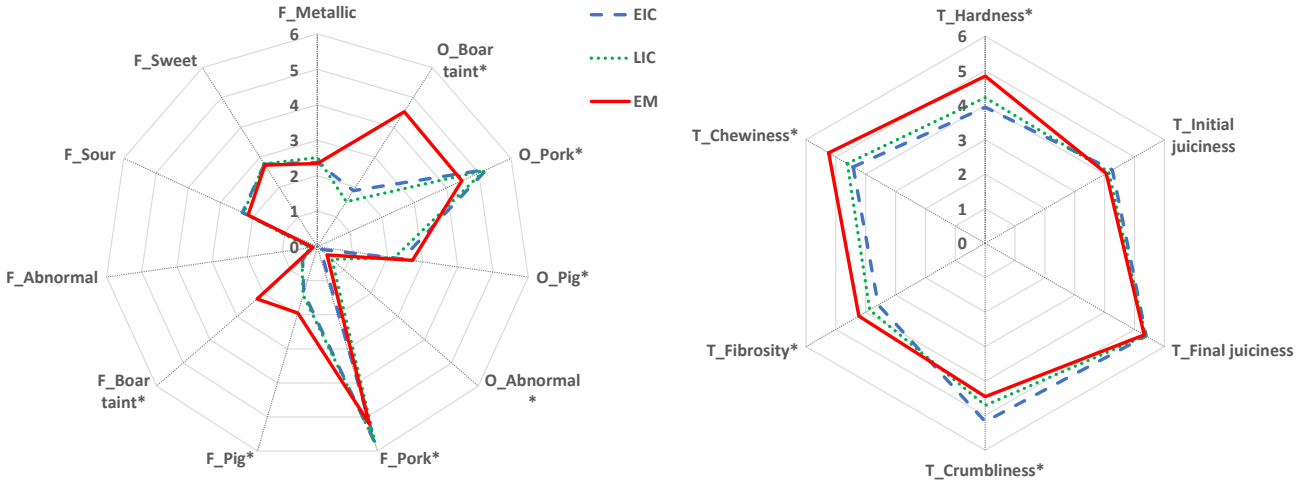
Two 1.5 cm thick loin slices with approximately 1 mm of subcutaneous fat were obtained from each pig. Each slice was cut into 5 pieces of 1 cm thickness perpendicular to the subcutaneous fat. The pieces were individually wrapped in aluminium foil and coded. They were then cooked in a preheated oven at 200ºC for 10 minutes to reach a core temperature of 72ºC. After cooking, they were kept warm until evaluation.

Sensory characterisation was carried out by 10 trained panellists in 11 sessions of 3 or 4 samples each. The order of presentation of the samples was designed to avoid the first sample and the carry-over effect. Attributes were selected in the training sessions from a list of attributes already used in other work. The attributes were rated on a continuous 10-point scale from 0 (low intensity) to 10 (high intensity).

III. RESULTS AND DISCUSSION

Figure 1a shows the odour and flavour scores by treatment. The greatest differences were found in the odour (O) and flavor (F) of the boar taint, which was significantly higher in meat from EM than in meat from EIC and LIC, which is consistent with several studies [1,2,3]. The boar odour of meat from EIC was significantly higher than that of meat from LIC (1.88 vs. 1.51). In agreement with this result, Zoels et al [2] reported higher androstenone and skatole levels in EIC pigs than in LIC pigs. Although the abnormal odour was very low, it was higher in LIC than in EIC, with EM in between. However, the abnormal flavour was not significantly different between the groups. EM meat had a higher pig odour and a lower pork odour and flavour. In terms of texture characteristics (Figure 1b), EM meat was harder, which is consistent with Font i Furnols et al [1]. However, in contrast to the earlier work [1], no

differences in juiciness were found between the sexes, which is consistent with the meta-analysis by Pauly et al [3]. Meat from EM also showed higher fibrosity and chewiness than meat from LIC and EIC. The crumbliness was higher in meat from EIC than in meat from LIC and EM.



(a) (b)

Figure 1. Odour and flavour (a) and texture (b) sensory attributes for loin from entire male (EM), early (EIC) and late (LIC) immunocastrated pigs (* after the attribute name indicate significant differences P<0.05).

IV. CONCLUSION

Under the conditions of the present study, immunocastration, both early and late, reduces the odour and flavour of boar taint in pork loin. The reduction in boar odour is slightly greater in loins from late immunocastrated pigs than in early immunocastrated pigs. Meat from entire male pigs is harder, more fibrous and less chewy than meat from immunocastrated pigs.

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THE ATTITUDES OF SERBIAN CONSUMERS TOWARDS CULTURED MEAT

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I. INTRODUCTION

Cultured meat, also known as lab-grown, cell-cultured, or in-vitro meat, represents a pioneering frontier in food technology aimed at producing meat products directly from animal cells without the need to raise and slaughter animals. The concept was first introduced in the early 2000s, with significant scientific interest sparked by the potential for reducing the environmental impact and ethical concerns associated with traditional animal farming [1]. Efforts are being made aiming, not only to scale up production and improve the texture and taste of cultured meat products, but also to make them cost-competitive with conventional meat by streamlining the manufacturing processes and sourcing more efficient growth mediums. As of the early 2020s, several products have approached regulatory approval stages in different countries, promising a future where cultured meat might become a common feature in supermarkets and restaurants worldwide, offering a sustainable alternative to traditional meat production [2]. The aim of this research was to reveal, for the first time,the attitudes of Serbian consumers toward cultured meat.

II. MATERIALS AND METHODS

The survey was conducted during 2024 through an online questionnaire directed at 450 consumers of the Republic of Serbia. The consumers were selected as previously described in Tomasevic et al [3] and collected as explained in Miloradovic et al [4]. Only the fully answered questionnaires (414) were considered for data analysis. A questionnaire containing two parts was developed to investigate consumer's attitudes towards cultured meat. The first part was about the main demographic characteristics of participants including sex, age, education, place of living and household incomes. The second part consisted of questions regarding their opinions and beliefs towards cultured meat. Because the data depicted here are only preliminary results of a much larger survey involving consumers from other countries, we have used only descriptive statistics for their presentation in this short manuscript.

III. RESULTS AND DISCUSSION

Out of 414 respondents, 59.4% were females, 39.1% were males and 1.4% preferred not to reveal their gender. All of consumers had some kind of educational background while majority of consumers (more than 60%) were highly educated, holding an university degree. Most of them (65%) were living in urban areas with more then 100,000 inhabitants, while a minority of 55 respondents (13.3%) lived in rural areas. Almost half of the consumers (43.7%) considered that

they are living well, but can only set a little money aside, while only 6.1% regularly or sometimes had difficulties in meeting daily costs in their households. More than 43% of the Serbian consumers had completely negative, while only 7% of them had completely positive attitudes towards cultured meat. The majority of Serbian consumers (288 or 65.3%) believed that cultured meat is also called “artificial meat” and 237 of them (53.7%), that it contains protein of animal origin. Very few (5%) believed that it contains only plant-based ingredients or that it is completely plant-based (3.8%). If they would have the opportunity to eat cultured meat, 37% of the Serbian consumers would do so because of curiosity. This is similar to the situation in Germany (38%) [6] or in Belgium (39.3%) [7], but much less welcoming compared to Brazil, where over 85% of the consumers were curious enough to do the same [8]. When it comes to motivation for eating cultured meat, Serbian consumers mostly mentioned environmental reasons (12.6%), animal welfare reasons (12.5%), reason to reduce meat consumption (9.7%), health reasons (3.9%) and other causes. When asked if cultured meat meets people’s nutritional needs, more than 42% of the Serbian consumers did not know, 16% thought it lacked some and another 16%, it lacked several important nutrients, while only 5.6% thought it was somewhat or much richer in nutrients compared to conventional meat.

IV. CONCLUSION

Consumer acceptance of cultured meat in a Serbian market is a complex and evolving issue, influenced by a variety of factors including health perceptions, ethical consideration and other issues. While the idea of meat grown from cells in a lab might initially seem off-putting to some,key motivators for acceptance include the potential for cultured meat to reduce the environmental footprint of meat production and diminish animal suffering. However, regarding Serbian consumers big challenges still remain, such as overcoming the "yuck" factor associated with lab-grown foods, addressing concerns about naturalness and processing, and ensuring that the products meet expectations for nutritional requirements.

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CONSUMER SENSORY EVALUATION OF MEAT FROM GROWING RABBITS FED DIET CONTAINING MULBERRY LEAVES

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I. INTRODUCTION

The nutritional profile of rabbit meat, when compared with other species, is characterized by relatively higher protein content, higher proportions of *n*-3 fatty acids, and low amounts of intramuscular fat, cholesterol and sodium, thus indicating that its consumption may provide health benefits to consumers [1]. With regard to the sensory characteristics, studies on consumer preferences found mixed results. People who consume rabbit meat think it has a pleasant delicate flavour and was tender. On the contrary, consumers who avoid rabbit meat judge it to have an unpleasant taste [2]. The main cause of refusal is its typical taste of wild meat, partially due to the fat content [3].

Morus alba is a fast growing, small to medium sized deciduous mulberry tree. Mulberry leaves are rich in nutrients and have a large number of unique bioactive substances, which can be used as high-quality livestock feed. Moreover, mulberry leaves are rich in aldehydes, alcohols, esters and ketones, which are the main characteristic compounds of meat flavour, so that feeding rabbits mulberry leaf could improve the composition of fatty acids in the meat, which in turn could enhance the flavour. Maintaining meat quality is essential to sustainable livestock management. Therefore, identifying alternative feed materials while considering consumer acceptance is crucial. Based on these considerations, this study was conducted to investigate the effect of the mulberry leaf meal supplementation in dietary concentrate on rabbit meat sensory quality.

II. MATERIALS AND METHODS

A total of 480 weaned, 45-days-old, crossbred rabbits (Hycole × Grimaud) were randomly allotted to 60 cages. All the rabbits were fed, *ad libitum*, the same commercial feed during the grower phase. For the finisher phase, two isonitrogenous, isolipidic, and isoenergetic dietary treatments were provided: 1) control diet (C) and 2) experimental diet including 10% of mulberry leaf meal (MLM). At 89 days of age, 30 animals/diet, representative of all the replications, were slaughtered. The *Longissimus thoracis et lumborum* (LTL) muscles were removed from both sides and used for meat proximate composition and sensory analysis. Water (W), crude protein (CP), ether extract (EE) and ash (A) contents were determined according to official AOAC methods. Sixty untrained assessors, regular rabbit meat eaters and free of food allergies, were recruited. The LTL muscle was cooked without salt or spice on a double plate grill to an internal temperature of 72°C and cut into 1.0 cm bite-size cubes. Samples were identified with a random three-digit number and randomly placed on a 2-compartment plate. Consumers were asked to rate the overall liking of meat and the liking of colour, tenderness, juiciness, and flavour, using the 9-point hedonic scale (1 = dislike extremely and 9 = like extremely). In addition, they were asked to assess, using the 5-point Just About Right (JAR) bipolar scale, the appropriateness of: colour (1 = too much light, 2 = too light, 3 = just about right, 4 = too dark, 5 = too much dark); tenderness (1 = too much tough to 5 = too much tender); juiciness (1 = too much dry to 5 = too much juicy); of flavour (1 = too much weak to 5 = too much strong). Before starting the test, consumers were given verbal instructions regarding the linear hedonic scale and the bipolar JAR scale and were asked to read and sign an informed consent form. The JAR results were combined with the overall liking scores and a Penalty Analysis (PA) was conducted to

highlight the attributes that have a high or significant impact on the overall liking. An attribute was considered significant when respondent percentage was higher than 20% and the penalty score (drop in overall liking) was higher than 1 [5]. Statistical analysis was performed using the XLSTAT Statistical Software (Addinsoft, New York, NY, USA). P values ≤ 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

Dietary MLM inclusion did not influence W, CP and A contents of meat, but led to lower EE when compared to the C meat (0.85% *vs* 1.21%; P < 0.01). As reported by Wang et al. [4], bioactive constituents from mulberry could repress fat deposition probably through elevating leptin-stimulated lipolysis. The Wilcoxon signed rank test showed no statistically significant differences for all the sensory attributes and overall liking between the two groups. In general, consumers liked the meat of both diets, as samples were rated above 6 ("like it slightly") with the only exception of the MLM meat colour (approximately 7 – "like it moderately") and the C meat juiciness (approximately 5 – "neither like it nor dislike it"). Most of the panellists rated C meat as JAR for colour (71.67%), tenderness (61.67%), and flavour (50%), while it was perceived as "too dry" for 76.67% of them. The most troublesome attribute of the C meat was tenderness. Indeed, 30% of the participants penalized it for being "too tough", with an overall penalty of 1.812 from the liking score (P<0.05). Regarding the MLM meat, the JAR frequencies of colour (75.00%) was similar to that highlighted for the C diet. The PA revealed that tenderness, juiciness, and flavour were the most troublesome attributes of the MLM meat. In fact, consumers strongly penalized it as being "too tough" (33.33% of answers), "too dry" (71.67%) and "too bland" in flavour (26.67%). The mean drops were significantly different from 0, and so was the overall penalty (1.077, 1.400 and 1.389 points from the liking score for tenderness, juiciness and flavour, respectively). Therefore, the meat from the MLM group exhibited lower levels of juiciness and flavour when compared to that of C group. In general, intramuscular fat positively influences meat sensory quality attributes, whereas a low amount of fat induces a less positive response.

IV. CONCLUSION

The fat content in rabbit meat was significantly reduced by inclusion of MLM in the diet. As a consequence, the MLM treatment slightly negatively affected the palatability characteristics of meat. However, this result has a less pronounced effect on product's overall quality.

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APPLICATION OF THE CHECK-ALL-THAT-APPLY (CATA) METHOD IN BEEF PRODUCED IN THREE DIFFERENT GRAZING SYSTEMS

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I. INTRODUCTION

The intercropping of grasses with pigeon pea (*Cajanus cajan* cv. BRS Mandarin) is an alternative to mitigate greenhouse gas emissions (GHG), increase forage availability throughout the seasons, enhance protein intake, and improve animal productivity [1]. Intensified systems and nutritional strategies enhance production efficiency and may play a significant role in the sensory quality of beef, influencing aspects such as flavor, aroma and texture perception. This study aimed to evaluate, using the Check All That Apply (CATA) method, the descriptive attributes of beef produced in three pasture systems by consumers.

II. MATERIALS AND METHODS

Twenty-seven *Nellore* steers, with an initial weight of 221±7 kg and aged between 15-16 months, were distributed into three treatments with three spatial replicates: degraded pasture (*Urochloa spp.*), recovered pasture (*Urochloa spp.* fertilized with 200 kg of N/ha^{-1}), and consortium pasture with pigeon pea (*Cajanus cajan* cv. BRS Mandarin) and *Urochloa spp*., a system used for mitigating GHG. The animals remained in the field for two years, after which they were slaughtered in a commercial abattoir. Samples of the *Longissimus thoracis* muscle (between the 12^{th} and 13^{th} rib) from the left half carcass of each animal were collected after 24 hours of chilling. Steaks 2.5 cm thick were aged for seven days at 0 – 2 ºC, seasoned with 1 g of salt, and cooked to an internal temperature of 75 ºC in a combined oven at 180 ºC. Subsequently, the meat samples were cut into cubes, wrapped in aluminium foil, and cooked at 60 ºC until the time of the test. The sensory test was approved by the Ethics Committee for Research with Human Subjects, under protocol CAAE 61386622.0.0000.5380, and conducted at Multiuser Laboratory for Sensory Analysis of Foods – LAMASA (FZEA/USP - Pirassununga/SP, Brazil) with the participation of 119 consumers. The samples were evaluated by the following descriptive attributes: "flavor" (intense, mild, grilled, greasy, rancid, metallic, bitter, sweet, and liver); "aroma" (intense, mild, grilled, greasy, rancid, and bloody); and "texture" (tender, hard, fibrous, juicy, dry, and liver) using the Check-All-That-Apply (CATA) method [2]. Each panelist evaluated the samples coded with a random three-digit number, in a balanced order [3]. The data were analyzed by correspondence analysis, Cochran's Q test and analysis of the presence and absence of the attributes using XLSTAT software.

III. RESULTS AND DISCUSSION

Cochran's test showed significant p-values ($p<0.10$) for intense beef aroma (IBAR), mild beef aroma (MBAR), intense beef flavor (IBFL), mild beef flavor (MBFL), greasy aroma (GARO) and grilled aroma (GRAR). This means that there were significant differences among the studied treatments. Multiple pairwise comparisons using the Critical difference (Sheskin) procedure showed that recovered and consortium pasture differed for mild beef aroma and grilled aroma attributes. The plot in Figure 1 allows us to verify the quality of the analysis, which is good (100%, with 72.99% for F1 and 27.01% for F2). Acceptability is associated ($p<0.001$) with the intense beef flavor attribute when it is present in the presence/absence analysis.

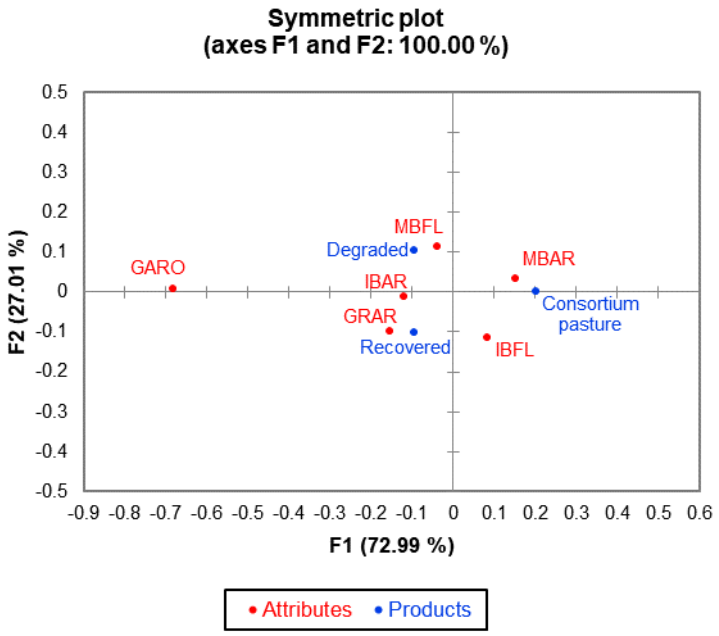

Figure 1. Descriptors of the sensory characteristics of beef produced in three pasture systems as determined by the CATA method. Attributes: GARO – Greasy aroma; GRAR – Grilled aroma; IBAR – Intense beef aroma; IBFL – Intense beef flavor; MBAR – mild beef aroma; MBFL – Mild beef flavor

IV. CONCLUSION

The studied pasture systems showed differences for intense beef aroma, mild beef aroma, intense beef flavor, mild beef flavor, greasy aroma and grilled aroma attributes. Intense flavor attribute affected acceptability of beef produced in the different systems. The consortium system of pigeon pea+*Urochloa spp*., in addition to being an efficient mitigation strategy for GHG emissions, produced meat with a milder beef aroma and less grilled aroma, in consumers' perception.

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IMPACT OF ELECTRICAL STIMULATION ON THE SENSORY PERCEPTION OF NORMAL CHICKEN BREAST FILETS AND IN FILETS AFFECTED BY WOODEN BREAST MYOPATHY

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I. INTRODUCTION

Promoting high weight gain quickly can result in meat quality problems. Thus, due to the rapid growth of broiler chickens lines to achieve greater meat yield muscle defects such as woody breast myopathy (WB) have emerged, which can affect the texture and quality of the meat of these birds [1, 2]. Electrical stimulation is a procedure that induces muscle contraction, improving glycolytic activity and influencing the drop in pH and *rigor mortis* [3], thus favoring the development of tender meat and may be an alternative to these muscular defects. Therefore, this study aimed to observe the influence of electrical stimulation on the tenderness and juiciness of normal chicken breasts and those affected by Woody Breast Myopathy.

II. MATERIALS AND METHODS

The electrostimulation device consisted of metal conductors with protection insulation fed by a controlling font Fluxo LFX-500. The potential difference between electric poles: feet = positive (+) and breast = negative (-) of 50V was applied at an alternating current of 20Hz for 50 seconds after the bleeding stage on Cobb 500 Male broilers from the same flock.

The sensory analysis was conducted by 50 untrained panelists, according to the 510/2016 Resolution by Brazil's National Health Council [4], after signing a declaration of free and informed consent.

Paired comparison tests [5] were performed in triplicate, in which the panelist would determine from two cooked samples which one was superior on the tenderness and juiciness attributes. To avoid influencing the tasters, the samples were randomly codified. Electrically stimulated breasts meat were compared with non-stimulated breasts meat, both normal (healthy) breasts and those affected by woody breast myopathy.

The results were subjected to analysis of variance and the difference between electrically stimulated and non-electrically stimulated breasts was tested with the Student's t-test, at a significance level of 5% ($P < 0,05$).

III. RESULTS AND DISCUSSION

The paired comparison tests suggested that normal electrically stimulated breasts displayed greater tenderness ($P < 0.05$) and greater juiciness ($P < 0.05$), than the non-stimulated ones. Out of the 50 panelists, 40 selected the stimulated samples as more tender and 33 as juicier. Tenderness is a multi-parameter sensory attribute and together with juiciness of meat, defines a large part the consumers' perception of overall quality [6]. Electrical stimulation of slaughtered birds accelerates the development of *rigor mortis*, and consequently, its resolution, inducing a faster pH fall and increasing the sarcomere length, resulting in increased tenderness [7]. Considering that in-mouth sensory tenderness is generally correlated to instrumental textural measurements [8], these results were in agreement with

those found in previous studies, that observed lower Warner-Bratzler shear force values in breasts electrically stimulated after bleeding [7, 9].
The Wooden Breast myopathy affects the texture of raw broiler breast filets, making it firmer upon palpation, with higher compression force compared to normal breast filets [10]. Hence, its occurrence reduces the quality and acceptance of both raw as well as cooked meat and meat products [11]. The application of electrical stimulation improved the texture of woody breasts, making them more tender than the ones that didn't receive the stimulus ($P < 0.05$), according to 39 panelists. However, in the attribute of juiciness, there was no significant difference ($P > 0.05$), with 28 out of the 50 panelists considering the stimulated samples as juicier and 22 the non-stimulated woody breasts.

IV. CONCLUSION

Low voltage electrical stimulation after bleeding resulted in improvements in sensory textural traits of cooked broiler filets. Normal breasts subjected to electrical stimulus developed greater tenderness and juiciness. Similarly, electrically stimulated wooden breasts displayed greater tenderness, although there was no significant difference in juiciness. Therefore, electrical stimulation can be a method to aggregate the quality and value of broiler meat products, as well as to minimize undesirable effects of wooden breast on final product quality.

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The impact of intramuscular fat grade and aging on consumers' evaluation of beef tenderness

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I. INTRODUCTION

Consumers evaluate overall satisfaction when consuming beef, considering tenderness, flavor, and juiciness, among other factors. Among these, tenderness is known to have the most significant impact [1-3]. For general consumers or untrained panels, the evaluation of beef taste/satisfaction is influenced by societal culture and previous experiences [4]. For example, Korean and Japanese consumers prefer tender beef with fine marbling, while European consumers prefer beef with less marbling for health reasons [5,6]. The tenderness of beef is determined by various mechanisms, both inherent and acquired, such as muscle fiber density, characteristics of connective tissue, level and distribution of marbling, post-slaughter processing, and aging [7]. Shear force is a common method for mechanically measuring the tenderness of meat [8]. Although shear force, a physical measurement of meat texture, does not fully represent the tenderness perceived by consumers [9], consumers typically classify beef with shear force <4.37 kg as tender and >5.37 kg as tough [10]. The study was conducted among South Korean consumers to compare the impact of intramuscular fat level and aging on tenderness evaluation.

II. MATERIALS AND METHODS

From 2007 to 2020, data on Hanwoo beef analyzed at Jeonbuk National University and the National Institute of Animal Science were collected. The analysis results for the Longissimus thoracis (LT) muscle from a total of 90 heads (7 bulls, 19 cows, 10 heifers and 57 steers) generated across four projects were utilized. All animals were conventionally raised in feedlots and slaughtered according to the regulations set forth by the Korea Animal Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs for beef slaughter at commercial abattoirs. And then immediately moved to a chilling room and stored at 4 °C, after 24 h in the chilling room, all carcasses were graded according to the Korean Beef Carcass Grading System included intramuscular fat grade [11]. Aging was initiated 24 hours post-slaughter, designated as Day 0 of aging. The shear force and sensory-tenderness evaluation methods used in the analysis were consistent across all projects. The methods used were as follows: Shear force measurements were conducted using a Warner-Bratzler blade. Meat blocks were heated in a water bath until the core temperature reached 70°C, then cooled in running water for 30 minutes. Cores(1.25cm) parallel to muscle fibers were took, and shear force was measured using an Instron Universal Testing Machine (Model 3342; Instron Corporation, Norwood, MA, USA) For sensory evaluation of tenderness, a 100mm line scale method was used, with the item scale ranging from very tough (0) to very tender (100). The data from samples with shear force values ranging between 3-5 kg out of the collected data were utilized for statistical processing. The statistical analysis was conducted using IBM SPSS Statistics (version 27.0, SPSS Inc., Chicago, IL, USA). The comparison of the impact of intramuscular fat level and aging on tenderness was conducted using multiple regression analysis.

III. RESULTS AND DISCUSSION

To explore how intramuscular fat grade and aging in the LT muscle of Hanwoo affect tenderness evaluations by Korean general consumers, a multiple linear regression analysis was conducted, and

the results are shown in Table 1. The analysis revealed F=21.996 (p<0.001), indicating the suitability of the regression model, with an Adj.R^2 of 0.300, explaining 30% of the variance. The results from shear force measurements, a mechanical quantification of meat tenderness in the LT muscle of Hanwoo with shear force values between 3-5 kg, showed that intramuscular fat grade had a significant impact on tenderness evaluations with β=0.500 (p<0.001). Similarly, aging also had a statistically significant impact on tenderness evaluations with β=0.332 (p<0.001). These results suggest that for intramuscular fat level, with β=3.311 (p<0.001), for each unit increase in intramuscular fat level, tenderness increased by 3.311 points. And for aging, with β=0.913 (p<0.001), for each day of aging, tenderness increased by 0.913 points. The relative impact of intramuscular fat grade and aging on consumer tenderness evaluations was compared through the standardized coefficients β values. The standardized coefficients β values for intramuscular fat level and aging were 0.500 (p<0.001) and 0.332 (p<0.001), respectively, indicating that intramuscular fat grade has a relatively higher impact on tenderness evaluations compared to aging.

Table 1 – Comparison of the Impact of Intramuscular Fat Level and Aging on Consumer Sensory-Tenderness Evaluation in the Longissimus Thoracis Muscle of Hanwoo (Shear Force 3-5 kg).

Variations	Unstandardized coefficient		standardization coefficient	t(p)	TOL	VIF
	β	SE	β			
(Coefficient)	3.918	3.417		12.851		
Intramuscular-fat grade	3.311	0.565	0.500	5.864***	0.981	1.020
Aging days	0.913	0.235	0.332	3.887***	0.981	1.020
F(p)			21.996***			
Adj.R^2			0.300			
Durbin-Watwon			1.975			

***P<0.001

ACKNOWLEDGEMENTS

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SENSORY TEST OF DIFFERENT PIG PRODUCTION SYSTEMS IN EUROPE

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I. INTRODUCTION

Comparison of pig production systems, i.e., husbandry conditions (space – enrichment), breed combinations, feeding regimes and slaughter methods, have been investigated in the EU project mEAT-Quality [1]. The eating quality was one of the parameters studied. To obtain the most accurate comparisons, the sensory tests of the meat were carried out in one laboratory ensuring that all samples were evaluated by the same panel. Especially differences in feed composition can influence the sensory attributes [2], and therefore muscle and lard were prepared and assessed independently in this study. The aim was to analyze the sensory quality in fat and meat from pigs raised in different production systems.

II. MATERIALS AND METHODS

136 boneless pork loins with a top layer of fat from females and castrates were used for the sensory test. The loins came from 3 European countries and represented 7 different production systems with approx. 20 loins per treatment. The loins were frozen prior to transportation and stored at -18°C upon arrival and until the start of the test. The loins were thawed 48 hours prior to the sensory test, and the day before the test, the lard and the rind were removed from the loin (*M. longissimus thoracis*). 10 panelists participated in the analyses and received two days of training (3 hours each day) prior to the test, during which the vocabulary was agreed upon. The training was conducted according to ISO standards [3]. The sensory tests were carried out according to the guidelines of the accredited descriptive analysis [4,5] in ISO certified facilities. The outer fat layer (closest to the rind) was cut into 2x2x10 cm bites, boiled for 15 minutes in salted water, and stored at 5°C for 24 hours. On the day of the sensory test, the loins were cut into 20 mm pork chops. The pork chops were pan fried at 170°C for 8 min, to a core temperature of 68-70°C. The pork chops were weighed before and after cooking for calculation of the cooking loss. The fat bites were heated for 2 minutes on a Klemgrill (180°C) and served with the corresponding pork chop on a heated plate coded with a three-digit number. The assessors used a 15 cm intensity line scale with 25 attributes for the meat assessment and 10 attributes for the fat. The differences between production systems were analyzed with a model using an ANOVA with Post Hoc Tukey test (participant+sample+ participant*sample) from RedJade [6].

III. RESULTS AND DISCUSSION

The 7 production systems represented in total 3 control systems (one from each country) and 1-2 experimental treatments per country. Thus, the difference in sensory quality between control and experiment can be compared within country, and the overall significance in the sensory quality of meat and fat between the 7 production systems were calculated.

Some of the attributes (hardness, chewing time, tenderness) were highly correlated, as were the attributes describing taste and/or smell. Table 1 lists the attributes that describe the main difference between production systems with the exact statistical differences. Figure 1 shows the differences for the same attribute between the 7 systems in a spiderweb plot. The average cooking loss is shown in Figure 2. The meat from production systems 2-1 and 2-2 had a significantly lower cooking loss, was juicier and with more visible fat in the cutting line compared to meat from the other production systems. More intense sweet taste led to a lower intense piggy flavor. The sensory attributes of the fat showed that the ranking between the 7 production systems deviated from the ranking based on visible IMF, thus the fat attributes rated independently contributed to the assessment of the effect from treatment.

Table 1 – Selected sensory attributes, describing the differences* between 7 different production systems.

Production system**	Visual IMF	Tender-ness	Juici-ness	Sweet Taste	Piggy Flavor	Fried meat Flavor	Fried fat Flavor	Fat crisp-ness
1-1	1.1 ^{a}	7.8 ^{a}	7.5 ^{ab}	4.0 ^{a}	3.3 ^{ab}	8.5 ^{ab}	9.4 ^{ab}	5.8 ^{ab}
1-2	1.0 ^{a}	8.0 ^{a}	6.9 ^{a}	4.1 ^{a}	3.6 ^{a}	8.3 ^{ab}	9.8 ^{b}	6.1 ^{a}
1-3	1.2 ^{a}	7.4 ^{a}	7.1 ^{a}	4.0 ^{a}	3.2 ^{abc}	8.3 ^{a}	9.4 ^{ab}	6.1 ^{a}
2-4	2.5 ^{cb}	5.8 ^{c}	8.2 ^{b}	5.7 ^{b}	2.0 ^{bc}	9.0 ^{ab}	8.3 ^{a}	4.2 ^{c}
2-5	3.0 ^{c}	7.3 ^{a}	9.1 ^{c}	6.4 ^{b}	1.9 ^{c}	9.6 ^{b}	9.8 ^{b}	4.7 ^{cd}
3-6	1.3 ^{a}	6.1 ^{bc}	7.3 ^{ab}	4.2 ^{a}	3.4 ^{a}	8.1 ^{a}	9.2 ^{ab}	4.8 ^{bcd}
3-7	1.5 ^{ab}	7.0 ^{ab}	7.1 ^{a}	4.4 ^{a}	3.6 ^{a}	8.5 ^{ab}	9.3 ^{ab}	5.7 ^{abd}

* Columns with different letters are significantly ($p<0.05$) different.
** First number represents country; second number represents treatment.

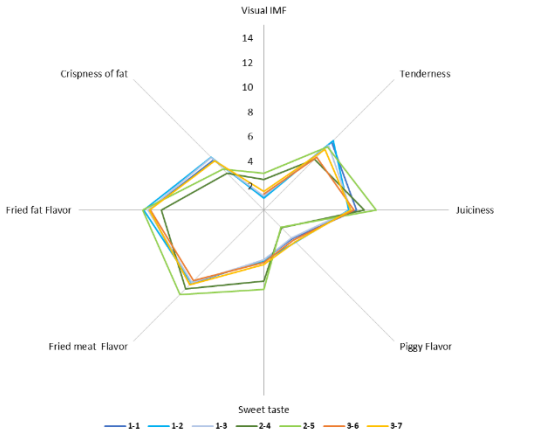

Figure 1. Spiderweb plot of sensory attributes from Table 1.

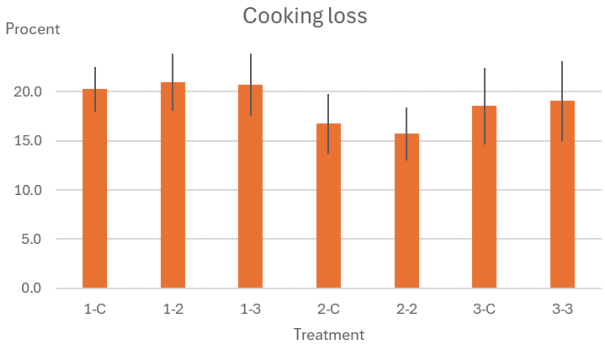

Figure 2. Average cooking loss measured in meat from the 7 production systems. The black line is the standard deviation (n=20).

IV. CONCLUSION

The sensory tests contribute to the understanding of differences between meat from pigs reared in different production systems. The sensory test of fat and meat prepared separately, but served together, provided some new nuances to the overall understanding of the eating quality of meat from different production systems.

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TESTIS WEIGHT IS A BETTER PREDICTOR OF BOAR TAINT THAN CARCASS WEIGHT OR AGE

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I. INTRODUCTION

Boar taint is an unpleasant urine- and faeces-like off-odour and off-flavour found mostly, but not exclusively, in the cooked meat of intact boars when they reach puberty but may also be found in gilts and castrates. Boar taint is mainly caused by androstenone (5α-androst-16-en-3-one), indole and skatole (3-methylindole) [1]. The exact onset of the taint varies depending on factors such as diet and genetics, but it usually occurs around the age of 6-8 months (24-32 weeks) for most boars. The option of slaughtering boars earlier than gilts is often considered and the maximum age mentioned is 25 weeks. Many countries will however use a maximum weight, rather than age, as the parameter of eliminating boar taint. Spain uses 105 kg, UK and the Netherlands 110 kg and Belgium and Denmark 115kg [2]. Weight and age as control mechanisms are not 100% successful to avoid boar taint in meat from intact animals and therefore online methods such human nose and more sophisticated methods such as mass spectrometry, Raman and biosensors are used to detect tainted carcasses [3]. This study investigated the effect of age, weight and testis size on the intensity of various pork fat odours to find an on-farm method to mitigate the risk of boar taint detected at abattoir level.

II. MATERIALS AND METHODS

A total of one hundred weaner male pigs (aged 28 days) were blocked according to weight and allocated to five treatment groups of 20 animals each which represented five different ages: 140 days, 147 days, 154 days 161 days and 168 days. The pigs were housed per group at a density of 1 m^2 per pig and grown on a commercial feed (Nova Feeds in Malmesbury, Western Cape, South Africa). Final live weights were recorded before slaughter. Both testes were weighted at slaughter to compare with sensory and analytical findings (androstenone; not reported in this study). Neck fat (500 g) samples were collected from warm carcasses, processed into sub-samples for skatole and androstenone analyses, as well as for sensory evaluation of aroma/odour of cooked fat. The samples were vacuum packed and stored at -70°C.

For sensory evaluation of pork fat odour, fat samples (1 cm^2), placed in 100 ml transparent glass ramekins and covered with thick aluminium foil, were cooked in an industrial oven (Hobart, Paris, France) at 180°C for 10 min [4]. The samples were cooled for 8 minutes and placed in ceramic mugs, covered with aluminium foil in a 70°C water bath for temperature control. A trained 12-member panel consisting of women evaluated the samples for 12 aroma/odour attributes previously deliberated and established during a training session (Table 1).

For this report multivariate statistical techniques were applied to elucidate patterns in data. Principal component analysis (PCA), employing the correlation matrix, was performed to determine the association between animal and carcass traits and sensory attributes using XLStat.

III. RESULTS AND DISCUSSION

Testis weight was positively (linear) correlated with slaughter age (R^2 = 0.523) and slaughter weight (R^2 = 0.681). All favourable odours were negatively correlated with testis weight, animal age and carcass weight. Unfavourable odours were positively related to testis weight, animal age and

weight (Table 1). Stronger relationships were recorded between testis weight and odour scores than between animal weight and age and odour scores (Table 1). Further testing of androstenone and skatole levels in relation to testis weight and odour scores will determine at what testis weight threshold would be needed to avoid the risk of boar taint. Bekaert et al. [5] showed that measurement of testis width and length and calculation of testis volume (volume=length^2×width×(π/6) can be used to determine testis size on live pigs on the farm. These measurements can be used as on-farm tools to determine and manage the risk of boar taint at abattoir detection level.

Table 1 – Linear relationship between sensory odour scores of pork fat and animal and carcass traits

	Mean sensory score*	R^2 value for linear regression		
		Testis weight	Animal age	Animal weight
Favourable odours				
Cooked pork meat	66.2	0.690	0.158	0.100
Fatty	69.6	0.114	0.035	0.020
Sweet-associated	38.3	0.492	0.115	0.066
Savoury	37.6	0.426	0.132	0.122
Grainy	35.1	0.204	0.023	0.010
Unfavourable odours				
Urine	18.8	0.848	0.024	0.048
Manure	5.3	0.769	0.072	0.074
Earthy	23.4	0.756	0.101	0.010
Moth balls	2.0	0.814	0.041	0.023
Sour	21.4	0.804	0.130	0.022
Sweat	12.6	0.807	0.237	0.035

*Sensory scale 0 = least intense; 100 = most intense

IV. CONCLUSION

Testis weight seems to be a better on-farm tool than animal age or weight to mitigate the risk of boar taint in carcasses from intact animals.

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UNDERSTANDING BRAZILIAN CONSUMER'S AWARENESS OF DRY-AGED BEEF

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I. INTRODUCTION

Consumer behavior towards meat products is constantly evolving, necessitating targeted studies to understand their intentions, habits, and attitudes [1]. Within the realm of gourmet meat products, dry-aged beef stands out as a notable focus. Therefore, assessing consumer understanding of dry-aged beef becomes crucial for expanding the market for this particular product. Completion testing, a qualitative methodology utilized in research studies, provides valuable insights into consumer perceptions and preferences regarding a specific product [2]. Hence, this study aimed to assess Brazilian consumers' perceptions of dry-aged beef and pinpoint positive and negative factors influencing their decision-making process in acquiring this product through completion testing.

II. MATERIALS AND METHODS

The data were collected via an online questionnaire designed in Portuguese using QualtricsXM software® and distributed through social media using snowball sampling. The study targeted meat consumers aged 18 and above to investigate their behavior regarding dry-aged beef. Ethical approval was obtained, and all participants provided consent. A total of 126 consumers completed the questionnaire, meeting the study's criteria. The questionnaire comprised 16 structured questions related to the consumption of dry-aged beef, with respondents indicating whether they had previously consumed it or not. Those who had consumed dry-aged beef were asked for further details regarding consumption location and frequency. For the completion test, participants were asked to complete two incomplete dialogues simulating a purchase intention [3]. The dialogues were to be completed with a positive association (eg. Yes, we should, because ...), explaining why they would purchase the product, and a negative association (eg. No, we should not, because ...), explaining why they would not purchase the product. For data analysis, responses were divided into two categories based on the frequency of dry-aged beef consumption: respondents who had consumed the product (RC) and those who had never consumed it (RNC). The data underwent statistical analysis using the chi-square (χ^2) test, with a significance level set at 95% ($P < 0.05$). Statistical analysis was conducted using the SPSS 24 software package (SPSS, Chicago, IL).

III. RESULTS AND DISCUSSION

During the completion test, 15 categories of terms were established to encompass the factors that influence the decision to purchase or not dry-aged beef (10 categories that encourage product purchase: "Curiosity", "Distinctive flavor", "Desire to try", "Special occasion", "Tenderness", "Low cost", "Simple preparation", "Healthy", "Trend" and "Good appearance", and 5 categories that restrict purchase: "High cost", "Poor appearance", "Unfamiliarity", "Apprehension" and "Strong flavor". The most mentioned categories by the RC and RNC groups included "Curiosity" (36.2%), "Distinctive flavor" (23.5%), "Desire to try" (14.7%), and "Special occasion" (13.1%). On the other hand, "High cost" (47.3%) and "Poor appearance" (39.5%) were the most cited factors in response to a negative

purchase intention, followed by "Unfamiliarity" (8.4%), "Apprehension" (3.0%), and "Strong flavor" (1.8%). These findings highlight the significance of high cost and visual perception in influencing consumers' decision-making regarding the purchase of dry-aged beef. The results of term category frequencies in the RC and RNC groups regarding positive purchase intention showed that only "Distinctive flavor" had no significant differences between groups ($P > 0.05$) (Figure 1). The term "Curiosity" was more mentioned by RNC (69%) than RC (31%), while all other categories were more frequent in the RC group, especially "Desire" (76%), "Special occasion" (84%), and "Tenderness" (94%). Additionally, category terms like "Simple preparation," "Low cost," "Healthy," "Trend," and "Good appearance" were exclusively mentioned by the RNC group. These findings suggest different priorities between the groups concerning dry-aged beef purchase, with the RC emphasizing sensory experiences and special occasions. Moreover, non-consumers (RNC) showed greater interest in exploring new experiences, particularly associated with "Curiosity." Regarding negative purchase intention, the RC group predominantly expressed concerns about "High cost" (66%), while the RNC group showed more concern about "Unfamiliarity" (80%) and "Poor appearance" (75%) (Figure 1). These results underscore the importance of perceived cost, familiarity, and appearance in the decision to purchase dry-aged beef.

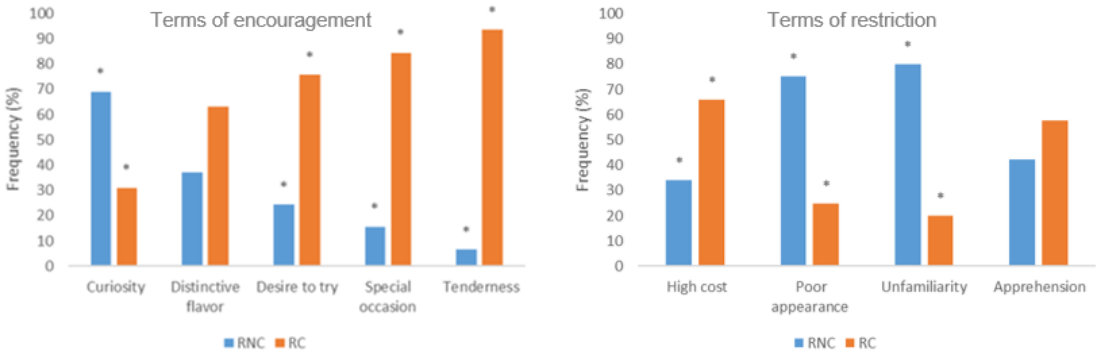

Figure 1. Frequency of term categories related to factors encouraging and restricting the purchase of dry-aged beef by consumers (RC) and non-consumers (RNC) in the completion test.

IV. CONCLUSION

Consumers in the RC group showed a more positive perception of the sensory attributes and emphasized the association of consuming this product with special occasions. This association was highlighted when considering the high cost of the product as a reason not to purchase it. On the other hand, the RNC group revealed a perspective of curiosity and unfamiliarity, expressing interest in exploring new experiences and flavors, as indicated by the terms "curiosity" and "never tried it". Thus, social influence might be a relevant factor in the decision to try new products. This points to an opportunity to educate and inform potential consumers about the attributes and benefits of this product.

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Influence of the Order of Presentation of Meat Samples on Consumers' Sensory Perception of Tenderness

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I. INTRODUCTION

Texture is an essential quality attribute of meat, playing a crucial role in consumer satisfaction and purchasing decisions. Previous studies have highlighted its importance, directly linking it to food texture and ease of chewing (OSÓRIO et al., 1998; MORTON et al., 2018). The ability to provide tender meat can significantly influence customer loyalty, as tenderness is often considered a determining factor in the choice of meat products. However, assessing tenderness is not without challenges; while objective methods based on physical and chemical tests are widely used, sensory analysis, though valuable, is susceptible to errors and subjective results. Individuals' willingness to judge samples may vary based on the sequence in which they are presented, highlighting the importance of considering and controlling this aspect in sensory study methodologies. (Jaeger, S. R., & Andrade, J. C., 2018).

This research aims to explore the influence of the order of presentation of meat samples on consumers' perception of tenderness. Specifically, we investigate whether the order in which samples are presented affects how consumers perceive the tenderness of the meat. This investigation is motivated by the need to better understand the factors that influence consumers' sensory perception regarding meat quality.

II. MATERIALS AND METHODS

Two pieces of *Longissimus thoracis* and two pieces of *Biceps femoris*, from Nelore breed cattle, were submitted, with cuts made parallel and perpendicular to the grain. These samples underwent heat treatment in an oven at a temperature of 180°C until reaching 70°C at the geometric center, controlled by a thermocouple. After being removed from the oven, the pieces were cut into 2 cm x 2 cm parallelepipeds, wrapped in aluminum foil, and kept warm in an oven at 60°C until serving time. A total of 120 regular consumers of beef were recruited to participate in the study. An affective acceptance test was conducted using a 9-point hedonic scale (1 – disliked extremely, 9 - liked extremely). The consumers were divided into two groups, each composed of 60 individuals. Each group was presented with four meat samples: two from the *Longissimus thoracis* group and two from the *Biceps femoris* group. For the first 60 tasters, the two *Longissimus thoracis* samples were presented first, namely, Parallel *Longissimus thoracis* Sample and Perpendicular *Longissimus thoracis* Sample, in random order, individually. Then, the analysis continued with the presentation of the *Biceps femoris* samples cut parallel to the grain, Parallel *Biceps femoris* Sample, and *Biceps femoris* cut perpendicular to the grain, Perpendicular *Biceps femoris* Sample. The same procedure was repeated for the other 60 participants, alternating the order of presentation of the meat samples, with the *Biceps femoris* samples being served first. To evaluate the results, the grades 1 to 9 were divided in classes, as follows: grades 1-4 were considered “I didn’t liked it”, grades 5-7 were considered “I liked it more or less” and grades 8-9 were considered “I really liked it”. The results were compared through an average test.

III. RESULTS AND DISCUSSION

According to the order of presentation of the pieces, the scores regarding tenderness changed significantly, as shown in Table 1.

Table 1 - Test for comparison of proportions, considering p-value <0.05

	Longissimus thoracis Par			*Longissimus thoracis* Per			*Biceps femoris* Par			*Biceps femoris* Per		
Tasters	0-60	61-120	**p value**	0-60	61-120	**p value**	0-60	61-120	**p value**	0-60	61-120	**p value**
"I didn't like it" (1- 4 grades)	20%	3.3%	0.0105	3.3%	0%	0.4758	28.3%	25.%	0.8365	23.3%	21.7%	1.00
"I liked it more or less" (5-7 grades)	48.3%	36.7%	0.2679	41.7%	31.7%	0.3436	48.3%	50.%	1.00	61.7%	50.%	0.27
"I really liked it" (8-9 grades)	31.7%	60%	0.0034	55%	68.3%	0.1887	23.3%	25.%	1.00	15.0%	28.3%	0.1209

The results of the present research clearly demonstrate the influence of the order of presentation of meat samples on consumers' perception of tenderness. For the initial participants (1-60), the *Longissimus thoracis* samples (Parallel *Longissimus thoracis* and Perpendicular *Longissimus thoracis*) presented first mainly received "I liked it more or less" ratings. However, when the subsequent participants (61-120) received the *Longissimus thoracis* samples last, there was a significant increase in "I really liked it" ratings, doubling in the Parallel *Longissimus thoracis* Sample from 31.7% to 60%, while proportionally decreasing the "I didn't like it" ratings from 20% to 3.3%. This increase can be attributed to direct comparison with the *Biceps femoris* samples, which were presented earlier and perceived as less tender.

Furthermore, we observed that the Perpendicular *Biceps femoris* samples, despite being naturally less tender, received more favorable evaluations when presented first to subsequent participants. This suggests that, in the absence of a direct comparison point, the samples were evaluated more positively. Under these conditions, the "liked extremely" ratings obtained higher values when presented first to participants 61-120, from 15% to 28.3%, which, although not significant by the comparison test, showed a notable increase.

IV. CONCLUSION

These results emphasize the importance of presentation order in consumers' sensory evaluation, highlighting the need for strategies to minimize comparison biases. The practical implications of these findings are significant for the industry, suggesting the importance of carefully planned presentation strategies to optimize consumers' perception of meat quality. Additionally, these results encourage further studies on how simple manipulations, such as presentation order, can influence the sensory evaluation of other food products.

ACKNOWLEDGEMENTS

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How working mothers choose between processed meat and vegetable products: A mixed-methods approach

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I. INTRODUCTION

The rising prevalence of adolescent obesity globally has been attributed to various factors, prompting efforts to combat this issue through strategies like providing nutritional information on food packaging [1]. Policies influencing food choices are crucial in addressing obesity, particularly among young individuals. Mothers who often make significant food decisions for their families can be influenced by front-of-package labels [2]. They encounter challenges in finding affordable and healthy options, such as processed meats, which are convenient but deemed unhealthy. This study aims to explore the link between working mothers and their subjective well-being in making choices regarding processed food products.

II. MATERIALS AND METHODS

620 women with children aged 10 to 18 were sampled using non-probabilistic online methods from June 2021 to December 2021. Various measurement tools were employed in the survey, including the Food Neophobia Scale, General Health Interest, Life Satisfaction Scale, Food-Related Life Satisfaction Scale, and Work-Family Balance Scale. A discrete choice model design was used to determine the factors influencing food choices, like ingredient count (Clean Label), portion size (Size), price (Price), nutritional warnings (Sodium NW and Fat NW), and protein source (Type). Cluster analysis and multinomial logit were conducted to categorize participants based on their responses to attributes of hamburgers like price, nutritional warnings, protein source, and package weight.

Descriptive statistics and factor analysis were conducted using JASP software, while Latent Gold software was employed for Latent Profile modeling. Profiles were established based on front-of-package attributes and sociodemographic data, with statistical criteria guiding selecting the most suitable model. The 4-class model was identified as the final choice, and a pairwise Wald test was executed to examine variables and covariates. Restrictions were imposed to enhance the model fit.

III. RESULTS AND DISCUSSION

Cluster 1, named Family-Oriented Plant-Based Mothers, represents the largest group with 48.72% of the total sample. This cluster shows a strong inclination towards plant-based food choices, minimal influence from nutritional warnings, and a preference for clean food labels, while mothers within this group adjust their selections based on their life satisfaction levels. Cluster 2, identified as Plant-Based Health-Conscious Mothers, displays a strong preference for plant-based options and a tendency to avoid nutritional warnings, particularly sodium and fat. Unlike the first cluster, this group is more inclined to make dietary choices based on their level of subjective well-being. Cluster 3, known as Meat Eaters Traditional Mothers, displays a preference for opting out rather than choosing the meat option. They show a stronger aversion to High Fat warnings compared to High Sodium warnings, and they highly value the Clean Claim attribute. This group, predominantly over 43 years old, consumes meat frequently but has lower Family Diet Followers and Life Satisfaction. Despite lower General Health Interest levels, they exhibit high food neophobia in both positive and negative aspects.

Cluster 4 comprises young immigrant mothers who are regular consumers of both meat and processed meat. This group shows a slight preference for higher prices (Price = 0.0051) and shows a positive attitude towards nutritional warnings, particularly favoring the "High Fat" warning (Fat NW = 0.3194) over the "High Sodium" warning (Sodium NW = -0.1577). This group exhibit the highest preference for larger package sizes (Size = 1.258) and are influenced by lower life satisfaction levels when making choices (SWL = -0.0876).

Table 1 – Summary of the choice model for the 4 class model.

	Wald	p-value	Class 1	Class 2	Class 3	Class 4
Class Size			0.4872	0.1921	0.2202	0.1005
Attributes						
Opt Out	52.3754	3.8e-19	-1.3831^{a}	0.6735^{b}	3.1245^{a,b}	-1.3570^{b}
Price	19.7314	0.0014	-0.0002	0.0000	-0.0013	0.0051^{a}
Type	122.5504	2.4e-27	0.2206^{a}	0.2206^{a}	-1.5310^{b}	-1.5310^{b}
Sodium NW	20.9684	0.00032	0.0271^{a}	-0.4797^{b}	-0.1356^{a,c}	-0.1577^{a}
Fat NW	37.0339	1.8e-7	-0.0145^{a}	-0.5822^{b}	-0.2527^{c}	0.3194^{a}
Size	14.4483	0.006	-0.0300	0.1059	-0.0065	1.2580^{a}
Clean Label	39.1868	6.4e-8	0.0691^{a}	0.1981^{a,b}	0.4656^{b}	-0.9818^{c}

Wald = Wald statistic; p-value = p-value for the Wald statistic; Different letters indicate significant differences between classes ($p<0,05$)

In between the Plant based (1 and 2) and the Meat based (3 and 4) clusters, there was a greater Health interest and self-reported diet interest, which concur with previous research [3]. Across the clusters, there was a dynamic between the NW, in these respect, the importance between each NW varies from cluster to cluster, which can be explained by the incapacity of the consumer to understand the difference between a product with one label, with two or without labels in respect of which is healthier [4].

IV. CONCLUSION

This study identified four distinct clusters of mothers based on their preferences and the influences of subjective well-being. Plant Based clusters were more influenced by subjective well-being. NW, claims and price had a greater impact in the Meat Based clusters. These findings support previous research suggesting a connection between health interest and dietary choices. Furthermore, the study highlights the varied influence of nutritional warnings across clusters, suggesting a need for targeted communication strategies based on consumer profiles.

ACKNOWLEDGEMENTS

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Brazilian Perspectives for Chicken Meat Production and Export

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I. INTRODUCTION

Chicken meat is a product of animal origin with high nutritional value and affordable cost, which is why it is widely consumed throughout the world. In 2023, more than 100 million tons were produced worldwide, despite the bird flu that affected several countries. The United States leads production with 21 million tons, although it is experiencing health problems involving millions of commercial birds. Brazil remains disease-free, according to the criteria of the World Organization for Animal Health (WOAH). However, migratory birds in transit may pose a health risk to domestic production. While affected countries try to control the spread of the disease, Brazil remains the second largest producer and leader in world exports. Based on statistical information were briefly analyzed the behavior of Brazilian production and exports of chicken meat in recent years, its main challenges and opportunities to maintain its position in the international market.

II. MATERIAL AND METHODS

The results were presented in the form of charts and tables from the survey of official statistics, class associations or other research sources.

III. RESULTS AND DISCUSSION

Chicken meat production in the United States had a growth of 3.0% in 2022, when compared to the year 2021. In the same period, Brazilian production grew by 1.4% and the country became the second largest producer in the world, since China showed a 2.7% reduction in production, corresponding to the third position. The European Union has remained virtually stable in its production, occupying the fourth place in the world. By the year 2023 growth in the United States was expected to be reduced to 1.3% due to health problems (SILVA et al., 2023). Indeed, growth did not reach 0.5%. Nevertheless, they continue to lead the world market. For Brazilian production, the prospect was of an increase of 2.1%, but growth was 2.5% (Figure 1). These numbers were disclosed by annual reports prepared by the Brazilian Association of Animal Protein (ABPA, 2024). The Brazilian projection for 2024 is to produce around 16 million tons, expecting that with two thirds of this volume will remain in the domestic market (BRASIL, 2023).

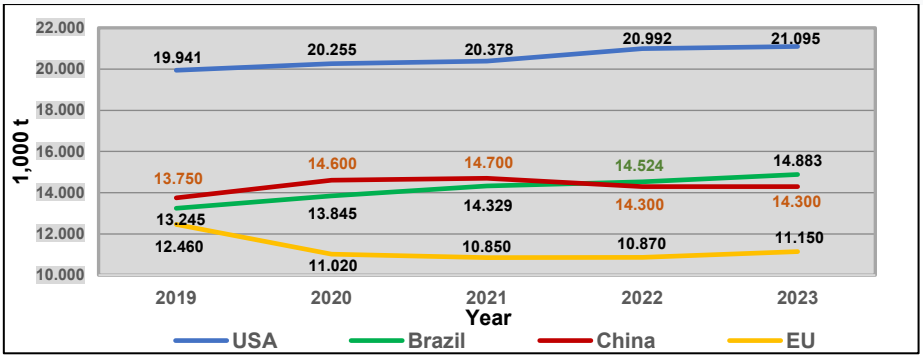

Figure 1. Top world producers of chicken meat in the period 2019-2023 (in million t/year).
Source: Elaborated by the authors with ABPA data (2024).

Table 1 shows the main countries that import chicken meat from Brazil. China is the largest destination for shipping. The volume of imports has decreased in recent years with the restrictions imposed by anti-dumping measures. With the cancellation of this measure by the Chinese government, shipments were resumed. The second largest importer is the United Arab Emirates, which has surpassed Japan in the

last two years. Brazil's ability to supply halal products has aroused the interest of Arab communities. Other important markets are Saudi Arabia and South Africa. These five countries together were responsible for shipping of more than 2 million tons in 2023 (ABPA, 2024).

Table 1. Main importing countries of Brazilian chicken (in tons/year).
Source: Elabored by the authors with ABPA data (2024).

Country/Year	Volume (t)			
	2020	2021	2022	2023
China	673,215	640,470	540,555	682,665
Japan	410,463	448,936	420,295	433,583
Un Arab Emirates	303,022	389,500	444,983	440,748
Saudi Arabia	467,546	353,584	340,127	376,953
South Africa	261,951	297,038	284,015	340,435

Figure 2 illustrates the evolution of Brazilian exports in the period 2019-2023. There is a continuous growth in the volume of exports during this period. In particular, in 2022 the growth was 5.9% in the volumes exported and 27.4% in revenue obtained. Then, the unit value went from US$1,662 to US$2,000 per ton of the product, representing an expressive increase of 20.3% compared to 2021, possibly influenced by the drop in production in China and the European Union. With a very favorable scenario for Brazil exports exceeded the volume of 5 million tons in the year of 2023 (ABPA, 2024).

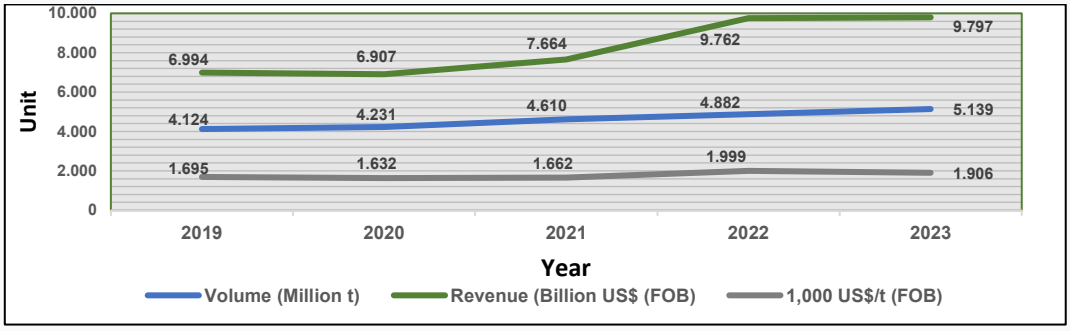

Figure 2. Evolution of Brazilian chicken meat exports in the period 2019-2023.
Source: Elabored by the authors with ABPA data (2024).

III. CONCLUSION

The poultry production chain continues to depend on the outbreaks of avian influenza in several countries. The global scenario favors Brazil, whose authorities have been maintaining preventive measures against the disease. There is a consistent demand for chicken meat due to its nutritional value, but the reduction in global production tends to increase consumer prices. Concerning Brazil, some factors may influence its position in the market, such as the balance between production and intern consumption, variations in exchange rates, implementation of new technologies, production costs, taxes, regulations, among other variables.

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Development of models of cooked hybrid products

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I. INTRODUCTION

Production and consumption of animal protein has more significant environmental and health impacts than plant proteins. Many researches emphasize the importance of meat alternatives and the use of plant proteins. However, the functionalities of these proteins, structure technique and sensory characteristics influence the properties, texture, nutritional value and acceptance of the final products. Despite recent advances and popularity in the market, continuous efforts and research are needed to make meat alternatives more sensorially attractive and with higher nutritional value [1]. The objective of this work was to investigate the use of some orphaned crops and other plant proteins in the development of new hybrid products, specifically, with 50% of total protein from lupine, broad bean, buckwheat, pea or soybean and the rest of protein from animal origin.

II. MATERIALS AND METHODS

Preliminary tests were carried out for the development of model system of cooked hybrid products using pork meat and vegetable protein derivatives: protein isolate (PI) of lupine, broad bean, pea and soybean; protein concentrate (PC) of broad bean, and buckwheat flour. The recipes of the nine batches manufactured are shown in Table 1.

Table 1 – Formulations of models of cooked hybrid products (g/Kg).

Formulations	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9***
Pork shoulder	900	450	450	450	450	450	450	450	450
Buckwheat flour	0	200	0	0	0	0	110	0	0
Broad bean PC	0	0	172	0	0	0	0	0	86
Lupine PI	0	0	0	115	0	0	0	0	0
Broad bean PI	0	0	0	0	119	0	0	0	0
Pea PI	0	87	0	0	0	115	100	0	58
Soybean PI	0	0	0	0	0	0	0	115	0
Salt	20	20	20	20	20	20	20	20	20
Water	80	243	358	415	411	415	320	415	386
Total (grams)	1000	1000	1000	1000	1000	1000	1000	1000	1000
Approximate Composition (%) **									
Proteins	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7
Starch - carbohydrates	0.0	12.3	0.3	0.0	0.0	0.0	6.7	0.0	0.15
Lipids	10.8	6.1	6.1	5.4	6.0	5.4	5.8	5.4	5.75
Fibers	0.0	1.7	2.6	0.0	0.0	0.0	0.9	0.0	1.3
Water	68.5	59.3	70.3	73.9	73.3	73.9	65.8	73.9	72.1

* F9 was prepared using 250g of F3 and 250g F6, mixed in the thermomix for 1 min.

** Based on information from the commercial product information data sheets.

The masses were produced in a Thermomix machine. Vegetable proteins with salt were added to ice water and mixed under stirring during one minute. Then the meat (previously minced in a mincer with a 4 mm hole plate) was added and there was new agitation during 1.5-2 minutes. Meat temperature: 1±1ºC, water temperature: 1±0.5ºC. The final temperature and pH of the mixture were measured (Table 2).

Table 2 – Temperature and pH of the mass mixtures.

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
Final temperature after the thermomix (ºC)	3.3	4.9	5.0	9.7	10.0	10.4	9.2	8.8	11.0
pH mixture	5.92	6.11	6.12	6.25	6.36	6.44	6.28	6.46	6.29

The resulting masses were vacuum packed and then placed in silicone molds to be cooked at 75 ºC (60 min.) in a Rational combined oven. The masses inside the molds were taken to a cold chamber (4-5 ºC). The texture when chewing or biting in the mouth (hardness/ firmness, pastiness, flouriness or crumbliness, springiness) were evaluated by 3 panelists, expert and trained on meat products according to ISO 8586:2023 [2] by consensus the next day after being heated in a microwave (900 watts) for 3 minutes (20 seconds per sample).

III. RESULTS AND DISCUSSION

Figure 1 shows the appearance of the samples after cooking.

Figure 1. Appearance of formulations (1 to 9 from left to right), after cooking 75 ºC/60 minutes.

F1, with 90% meat (2% salt, 8% water), presented color, firmness, stiffness and bite resistance characteristics of a meat product; F2, with 20% buckwheat flour and 8.7% pea protein isolate, showed high adhesiveness, a flour-like texture, adhering to the teeth and was a bit astringent; F3**,** with 17.2% protein concentrate of broad bean, showed good juiciness, was softer than F1, not being floury; F4, with 11.5% lupine protein isolate, was less firm than the F3; F5, with 11.9% broad bean protein isolate was softer that F4; F6, with 11.5% pea protein isolate, showed texture similar to F3; F7, with11,0% buckwheat flour and 10% pea protein, presented astringency (greater than F2), and a softer texture than F1; F8, with 11.5% soybean protein isolate, presented greater bite resistance, a slightly floury texture, soft texture); F9, with broad bean protein concentrate and pea protein isolate, had a brittle and floury texture.

IV. CONCLUSION

The main objective of this preliminary test was to select vegetable proteins to continue the studies of hybrid cooked product prototypes on a pilot scale, similar to frankfurter sausages and mortadella. The proteins with the best performance were protein isolate (PI) of broad bean and pea, and protein concentrate (PC) of broad bean.

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AMINOACID AND SENSORY PROPERTIES OF ALPACA MEAT (*Vicugna pacos*) PROCESSED BY SOUS VIDE TECHNOLOGY

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I. INTRODUCTION

Sous-vide is applicable to almost all type of foods. In sous-vide meat is generally cooked for a long time at 55–80 °C. In relative low temperature juiciness of meat is maintained while the flavour and tenderness are improved [1]. Cooking process highly impact the properties of food which are relevant to consumer preferences such as aroma, flavour, colour, chewiness etc. cooking not only changes food properties but also make food free from pathogens. Cooking also affects the nutritional value of food either positively or negatively. Traditional cooking uses high-temperature which contributes to a loss of nutritional components, flavour and colour etc. Amines can be categorized based on the structure of the precursor amino acid (aliphatic, aromatic, and heterocyclic) and the number of amino groups (monoamines, diamines, and polyamines) [2]. The consumer believes that meat is a choice for healthier diet because of high protein, low fat and low cost [3]. Meat is certainly a nutritious food and it is worth to be explored in sous vide application to be served as a ready-to-eat product [4]. Alpacas represent an important meat resource for rural Andean families [5]. Therefore, the aim of this study was to contribute to the knowledge of the amino acid and sensory characteristics of alpaca meat (*Vicugna pacos*).

II. MATERIALS AND METHODS

The alpaca meat (Leg) was cut into cubes of 5 cm, square to then be vacuum packed, to submit to different cooking treatments by sous-vide, later the composition of Amino Acid Analysis Using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC and sensory analysis were evaluated. The T1=Raw meat; T2=60°C/2h.; T3: 60°C/4h.; T4: 80°C/2h; T5: 80°C/4h. The values are expressed as mean ± S.E.M. (n = 3). Means with different superscript letter are significantly different ($P < 0.05$).

III. RESULTS AND DISCUSSION

Table 1. The amino acid composition, of alpaca meats samples, profile among all batches are possibly due to the different sources of amino acid. In relation to the arginine profile, except for some minor exceptions, individual amino acids of the two treatments T3 and T5 showed significant difference, although they showed significantly different amounts, of 142.87 and 160.27 (mg/100 g.), respectively. These differences were mainly attributed to the differences found for Leucine, followed by glutamic acid and serine, which were quantitatively more affected by the substitution of either alpaca meat. In this respect, amino acid composition of T2 and T4 was significantly lower than other meats, with amount at the end of cook. Was clearly reflected in the final product values, with significantly higher total values for meats.

Table 1. Amino acid profile (mg/100 g.) and nutritional significant ratios of different treatments.

Amino acids	T1	T2	T3	T4	T5
Arginine	65.22±5.93^{c}	117.12±22.06^{b}	142.87±20.27^{ab}	141.27±4.71^{ab}	160.27±3.43^{a}
Leucine	41.22±4.20^{dc}	68.81±12.96^{c}	86.64±14.28^{b}	92.13±5.05^{b}	111.72±1.23^{a}
Valine	34.39±1.97^{d}	50.69±9.10^{c}	61.22±7.74^{b}	65.52±1.59^{b}	77.61±3.18^{a}
Isoleucine	18.98±1.30^{d}	31.37±7.32^{c}	41.04±7.61^{b}	43.03±2.14^{b}	54.48±1.56^{a}
Phenylalanine	20.22±1.69^{d}	32.96±5.91^{c}	42.29±8.16^{b}	43.69±2.06^{b}	53.34±1.18^{a}
Lysine	34.45±5.45^{d}	53.58±6.17^{c}	58.77±5.46^{bc}	63.54±5.89^{ab}	71.79±0.47^{a}
Threonine	15.43±1.58^{d}	26.51±5.86^{c}	35.77±6.56^{b}	36.94±1.52^{b}	45.46±1.62^{a}

Methionine	16.67±1.21^{d}	25.80±3.77^{c}	30.88±4.54^{c}	32.15±1.17^{ab}	36.99±1.33^{a}
Histidine	29.43±2.26^{b}	37.02±2.52^{a}	38.03±4.19^{a}	37.01±0.26^{a}	40.02±2.07^{a}
Glutámic Ac.	56.57±5.34^{d}	100.09±22.08^{c}	144.18±28.85^{b}	152.42±9.58^{b}	193.70±4.71^{a}
Aspártic Ac.	20.97±2.18^{d}	38.90±9.81^{c}	58.93±11.31^{b}	65.64±3.71^{b}	85.31±2.89^{a}
Serine	36.85±6.00^{d}	71.31±13.60^{c}	93.20±20.26^{b}	96.76±5.66^{ab}	117.14±3.09^{a}
Alanine	28.11±1.92^{d}	49.69±8.06^{c}	62.43±10.39^{ab}	60.39±2.57^{bc}	72.37±0.31^{a}
Glycine	58.58±4.13^{c}	122.66±6.36^{a}	117.20±12.30^{a}	89.48±0.62^{b}	97.74±4.51^{b}
Tyrosine	18.88±2.57^{c}	30.74±6.08^{b}	40.71±8.24^{a}	39.23±0.79^{ab}	47.71±1.94^{a}
Cysteine	ND	ND	ND	ND	ND
Proline	9.28±8.68^{a}	10.19±17.65^{a}	ND	ND	ND

T1=Raw meat; T2=60°C/2h.; T3: 60°C/4h.; T4: 80°C/2h; T5: 80°C/4h. The values are expressed as mean ± S.E.M. (n = 3). Means with different superscript letter are significantly different (P < 0.05).

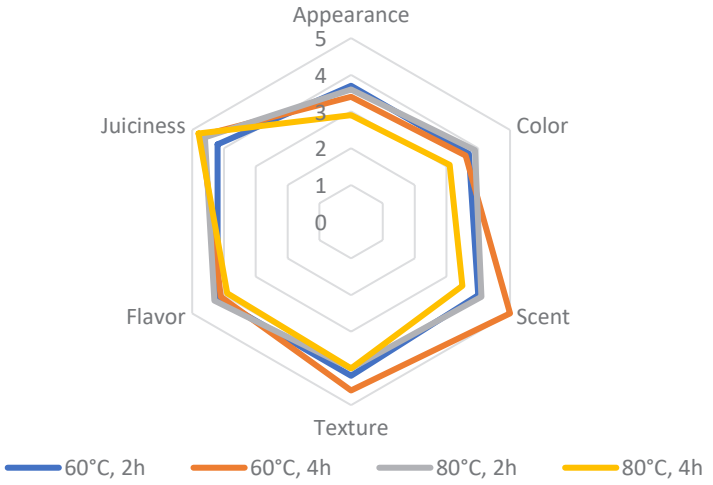

Figure 1. Sensory evaluation of meat treatments by sous-vide.

IV. CONCLUSION

The effect of temperature and cooking time using sous vide technology showed significant effects on amino acid profile, where a temperature increases. An effect was shown in the sensory evaluation of alpaca meat through sous vide technology at long times and low temperatures where it was possible to observe that the sample 60°C/2h and 80°C/2h treatments are the best in scent and color; in texture and flavor the best treatments are 60°C/4h. Regarding the juiciness of the 80°C/4h treatment was the best and in appearance.

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