



INTERNATIONAL SEMINAR:

ArtiSaneFood – Biopreservation and Risk Modelling
Approaches

BOOK OF ABSTRACTS

Edited by:

Ursula Gonzales-Barron

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ArtiSaneFood – Biopreservation and Risk Modelling Approaches: Book of Abstracts

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Preface



Dear Colleagues,

It is our great pleasure to welcome you in Bragança, Portugal, for the International Seminar “ArtiSaneFood – Biopreservation and Risk Modelling Approaches”, which is organised as the final scientific event of the PRIMA-funded project “Innovative Bio-interventions and Risk Modelling Approaches for Ensuring Microbial Safety and Quality of Mediterranean Artisanal Fermented Foods (2019-2023)”. The core objective of the ArtiSaneFood International Seminar is to present to the scientific community the project’s main results, which deals with the application of natural preservatives and biocontrol interventions for improving the safety of artisanal fermented foods produced in the Mediterranean. The International Seminar is also intended to provide a forum for the exchange of results from other ongoing research projects; and for the identification of research needs and pathways for ensuring safety, quality and recognition of artisanal, traditional and regional foods.

The ArtiSaneFood International Seminar aims to bring together researchers holding a genuine interest in the creation of the ArtiSaneFood *nucleus platform* that could promote and sustain the development of future industry-driven national and international projects, in the wider context of ensuring quality, valorisation, nutrition, beneficial health effects and societal impact. The International Seminar reunites researchers in the fields of food preservation and processing, chemistry of extracts, functional foods, biotechnology, microbiology, molecular biology, and predictive modelling. The scientific programme includes lectures, technical communications and e-poster presentations allocated in the following sessions:

- o Bio-preservatives as agents to ensure quality of artisanal foods;
- o Bioprotective lactic acid bacteria in artisanal foods;
- o Microbiome characterisation of fermented foods;
- o Challenge testing and predictive modelling in artisanal foods; and
- o Miscellaneous topics on quality of traditional foods

We, as food researchers based in a Mediterranean mountain region, are aware that the production of artisanal foods plays a key role in the development of rural regions, since the agricultural commodities used as raw materials are generally produced locally, allowing and stimulating local commercialisation and employment. It was therefore inspiring for us to have received a total of 70 submissions from researchers concerned about and actively working towards the valorisation of Mediterranean traditional foods.

We look forward to lively discussions, and hope that this meeting will give all of us the opportunity to share ideas, strengthen cooperation, and engage in future research and divulgation endeavours such as the ArtiSaneFood *nucleus platform*.

Warm regards,

Ursula Gonzales-Barron, the Project Coordinator.



Programme

24th May 2023

| Time | Sessions | Invited speaker |
|--------------|---|---------------------|
| 9:00 – 9:10 | Welcome session (President of IPB, Coordinator of CIMO, PRIMA Project Coordinator, ISBST-UMA Communication Manager) | |
| 9:10 – 9:40 | Session I: Bio-preservatives as agents to ensure quality of artisanal foods Keynote lecture: “Antibacterial and antioxidant activities of Mediterranean plant and industrial coproducts” | Nourhene Boudhrioua |
| 9:40 – 11:00 | Session I: Bio-preservatives as agents to ensure quality of artisanal foods Moderators: Halima Elhatmi and Ursula Gonzales-Barron 10-min oral presentations <ul style="list-style-type: none"> ○ Hanine Hached et al. Potential of Essential Oils from Eucalyptus, Peppermint and Pine as Food Preservatives ○ Ines Essid et al. Using Byproduct Extracts to Improve the Quality of Traditional Meat and Fish Products ○ Tatiane Oliveira et al. Evaluation of the Preservative Capacity of Brassica oleracea var. Acephala Extract in a Bakery Product ○ Zioud Amira et al. Antioxidant Activity of Garlic Powder During Storage ○ Yosra Zbiss et al. Extraction Optimisation of Phenolic Compounds through Response Surface Methodology from <i>Ficus carica</i> L. Leaves and Bioactive Evaluation ○ Cristiano Mateus et al. Stability Studies of Chestnut Flower-Based Ingredient Prospecting Incorporation into Beverages | |
| 11:00– 11:15 | Coffee break | |
| 11:15– 12.45 | e-Posters Miscellaneous Moderator: Ana Sofia Faria 4-min flash presentations <ul style="list-style-type: none"> ○ Sanaa Benassila et al. Impact of the Incorporation of Thyme and Rosemary Essential Oils on the Physicochemical Characteristics of Fresh Cheese ○ Ramla Khiari et al. Phytochemical Content, Antioxidant and Antibacterial Activities of Grape Marc Extracts from Chardonnay and Syrah Varieties Grown in Tunisia ○ Marwa Zeddini et al. Antimicrobial Potential of Eucalyptus Essential Oils ○ Mariem Ben Abdallah et al. Effect of Different Extraction Methods on Hesperidin Content, and in vitro Antioxidant and Antibacterial Activities of Ethanolic Orange By-Products Extracts ○ Mariem Zanzan et al. Exploring the Effects of Rosemary and Thyme Essential Oils on Merguez Sausage Fermentation: Physicochemical, Microbiological and Sensory Properties ○ Souhaieb Chrigui et al. Antibacterial and Antioxidant Activities of Methanolic and Decocted Extracts of <i>Salicornia arabica</i>: a Halophyte Plant Growing in Tunis Sebkh | |

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| | <ul style="list-style-type: none"> ○ Amal Dbeibia et al. Phytochemical contents, Antioxidant and Antibacterial activities of date seeds extracts from Tunisian Deglet Enour variety ○ Malek Ben Zid et al. Phytochemical Content, Antioxidant and Antibacterial Activities of Date Fruit Powder from Deglet Nour Variety ○ Yara Loforte et al. A Meta-Analysis of the in vitro Inhibitory Effects of Lactic Acid Bacteria Isolated from Dairy Products against Food-borne Pathogens ○ Abdelkhaleq Elmoslih et al. Biocontrol of <i>Listeria monocytogenes</i> CECT 4032 in milk, using bacteriocin-producing strain <i>Enterococcus mundtii</i> A2: contamination time effect ○ Salud Serrano et al. Microbiological and Genetic Study of <i>Listeria monocytogenes</i> Biofilms after their Transfer to Cooked Ham and Simulated in vitro Digestion ○ Gisela Rodrigues et al. Thermal Inactivation Kinetics of <i>Salmonella</i> Typhimurium in <i>Alheira</i> Sausage Batter ○ Nouha Harizi et al. Effect of Ebeam Irradiation on the Antioxidant and the Antimicrobial Activities of Defatted Freeze Dried Cow and Camel Milk Fractions ○ Beatriz Silva et al. Effect of Herbal Extracts on the Survival of <i>Staphylococcus aureus</i> in Goats' Raw Milk Cheese ○ Ibtissem Ben Hmidene et al. Exploring the Inhibitory Potential of Capsicum Annum Extracts Against <i>Listeria monocytogenes</i> and Spoilage Bacteria in Fresh Beef Sausages During Storage ○ Jrad Zeineb et al. Potential Effects of Syrup and Seeds Powder of Tunisian Pomegranate (<i>Punica granatum</i> L.) on Characterisation and Sensory Properties of Camel Milk Yogurt ○ Frédérique Pasquali et al. Genomic Snapshot of Foodborne Pathogens from Artisanal Food Productions of Animal Origin in the Mediterranean Region: Occurrence, Resistome and Virulome ○ Valentina Indio et al. Metagenomic Investigation of Artisanal Fermented Meat from the Mediterranean Area | |
| 12:45 – 14:00 | Lunch | |
| 14:00 – 14:30 | Session 2: Bioprotective lactic acid bacteria in artisanal foods Keynote lecture: “ Bioprotective potential of autochthonous lactic acid bacteria in Moroccan Jben cheese and Merguez sausage ” | Fouad Achemchem |
| 14:30 – 16:15 | Session 2: Bioprotective lactic acid bacteria in artisanal foods Moderators: Antonio Valero and Ines Essid 10-min oral presentations <ul style="list-style-type: none"> ○ Sofia Faria et al. Assessment of the Bioprotective Capabilities of Lactic Acid Bacteria Isolated from Artisanal <i>Alheira</i>, a Portuguese Fermented Sausage ○ Wafa MkaDEM et al. Biocontrol of <i>Listeria monocytogenes</i> in fermented milk by selected <i>Lactocaseibacillus paracasei</i> and effect of citrus peel extract on its survival ○ Nathalia Fernandes et al. Meta-Analysis of the Inhibitory Effects of Indigenous Lactic Acid Bacteria Supernatant from Dairy Origin against Foodborne Pathogens | |

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| | <ul style="list-style-type: none"> ○ Olga Bonilla et al. Exploring the Technological and Safety Properties of Lactic Acid Bacteria Isolated from Artisanal <i>Salchichón</i>: in Search for a Novel Starter ○ Kaoutar Boussif et al. Effect of Commercial Starter Cultures and Selected Bacteriocinogenic Lactic Acid Bacteria on the Microbiological Quality of Goat <i>jbén</i> Cheese ○ Javier Sánchez Martín et al. Study of the Bioprotective Potential of Three Different Lactic Acid Bacteria Cocktails against <i>L. monocytogenes</i> in Vacuum-Packaged Cold Smoked Rainbow Trout ○ Youssef Ezzaky et al. Impact of Commercial Starter Cultures on the Microbiological and Physicochemical Properties of Traditional <i>Merguez</i> Sausages ○ Jonata Ueda et al. Effect of the Drying Process and Incorporation of Natural Preservatives in the Viability of Lactic Acid Bacteria in Yogurts | |
| 16:15 – 16:30 | Coffee Break | |
| 16:30 – 18:30 | Last annual meeting of the ArtiSaneFood consortium (WP leaders) | |
| 19:30 – 23:00 | The Artisans Party | |

25th May 2023

| Time | Sessions | Invited Speaker |
|---------------|---|------------------------|
| 9:10 – 9:40 | Session 3: The microbiome of fermented foods Keynote lecture: “ Exploitation pathways of the microbiome characterisation of fermented foods ” | Alessandra de Cesare |
| 9:40 – 10:50 | Session 3: The microbiome of fermented foods Moderators: Yosr Haffani and Fouad Achemchem 10-min oral presentations <ul style="list-style-type: none"> ○ Nathalia Fernandes et al. Genomic and Phenotypic Analysis of Lactic Acid Bacteria Isolated from <i>Alheira</i>, a Portuguese Traditional Fermented Sausage ○ Olga Bonilla et al. Integration of Contributing Factors Affecting Microbiological Diversity and Food Safety throughout the Artisanal <i>Salchichón</i> Manufacturing ○ Láís Carvalho et al. Phylogenetic Analysis of Lactic Acid Bacteria species from Cheese: a Comparison between Taxonomy and Physicochemical Characteristics ○ Wafa Mkadem et al. Safety Evaluation of Lactic Acid Bacteria and its Bacteriocin Production Based on Whole Genome Sequencing ○ Olga Bonilla et al. Biofilm-forming Ability of <i>Listeria monocytogenes</i> and <i>Staphylococcus aureus</i> strains isolated from Artisanal Fermented Products Processing | |
| 10:50 – 11:10 | Coffee Break | |
| 11:10 – 11:40 | Session 4: Predictive modelling in artisanal foods Keynote lecture: “ Utility of dynamic modelling for ensuring microbiological safety of artisanal foods ” | Ursula Gonzales-Barron |
| 11:40 – 12:10 | Session 4: Predictive modelling in artisanal foods Keynote lecture: “ Conduction of tailored challenge testing experiments in artisanal foods. Data preparation for modelling purposes ” | Antonio Valero |

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| 12:10– 13:45 | Lunch | |
| 13:45 – 15:45 | <p>Session 4: Predictive modelling in artisanal foods Moderator: Vasco Cadavez and Sihem Bellagha 10-min oral presentations</p> <ul style="list-style-type: none"> ○ Mariana Pimentel et al. Estimation of Growth Parameters of <i>Listeria monocytogenes</i> under Different Conditions of Mexican Oregano Essential Oil (<i>Poliomintha longiflora</i> Gray), pH and NaCl ○ Ibtissem Ben Hmidene et al. Assessing the Efficacy of Bioactive Mint Extract in Controlling <i>Listeria monocytogenes</i> Growth during Fermentation and Drying of Traditional Dried Sausages: a Mathematical Modelling Approach ○ Ahmed Elidrissi et al. Quantifying and Modelling the Bioprotective Effect of <i>Latilactobacillus sakei</i> AE127, <i>Lactococcus lactis</i> AE89, and <i>Enterococcus mundtii</i> AE24 Against <i>Listeria monocytogenes</i> 7467 on Vacuum-Packed Cold-Smoked Moroccan Trout. ○ Beatriz Silva et al. Omnibus Modelling to Describe the Effects of Thermisation on <i>Staphylococcus aureus</i> in Goat's Raw Milk ○ Youssef Ezzaky et al. Oregano Essential Oil and Processing Conditions: A Predictive Model for Effective <i>Salmonella</i> spp. Inactivation in Sausage Medium ○ Gisela Rodrigues et al. Microbiological and Physicochemical Quality Categorisation of Artisanally Produced <i>Alheira</i> Fermented Sausages in Northern Portugal ○ Olga Bonilla et al. Assessing the Impact of Starter Cultures on the Behaviour of <i>Salmonella</i> spp. and <i>Listeria monocytogenes</i> in Dry-Cured Fermented Sausages ○ Laires Lima et al. Comparative Study of Hybrid Optimisation Methods in the Extraction of Antioxidant Phytochemicals from Grape Seed (<i>Vitis vinifera</i>) ○ Mariana Pimentel et al. Effect of Mexican Oregano Essential Oil (<i>Poliomintha longiflora</i> Gray) on the Thermal Inactivation of <i>Listeria monocytogenes</i>: An <i>in vitro</i> essay | |
| 15:45 – 16:15 | <p>Session 5: Miscellaneous topics on quality of traditional foods Keynote lecture: “Risk assessment of artisanal food products: importance of monitoring process parameters and microbiological quality”</p> | Laurent Guillier |
| 16:15 – 16:30 | Coffee Break | |
| 16:30 – 17:50 | <p>Session 5: Miscellaneous topics on quality of traditional foods Moderators: Rafika Ben Chaouacha Chekir 10-min oral presentations</p> <ul style="list-style-type: none"> ○ João Gonçalves et al. Bio4Drinks Project: the Obtention of Natural Multifunctional Ingredients for the Beverage Industry ○ Sofia Faria et al. Evaluation of the Influence of Intrinsic Properties on the Microbiological Quality of Traditional Portuguese <i>Transmontano</i> Hard Cheese made from Raw Goat's Milk ○ Regina Soares et al. Effect of Chestnut Flower Extract on the Flavour Stability of Craft Beers | |

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| | <ul style="list-style-type: none"> ○ Oussaief Olfa et al. Physicochemical properties and biological activities of fermented camel milk: A comparative study with fermented cow milk ○ Izamara Oliveira et al. Magnesium and Manganese Induced Changes in Chemical, Nutritional, Antioxidant and Antimicrobial Properties of Pansy and Viola Edible Flowers ○ Yosr Haffani et al. Anti-Tumoral Properties of <i>Allium roseum</i> on Breast Cancer Cells | |
| 17:50 – 18:10 | Presentation of the ArtiSaneFood e-platform for regional food producers | Vasco Cadavez |
| 18:10 | Closure of the project. Farewell | |



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The ArtiSaneFood Project

The ArtiSaneFood project “Innovative Bio-interventions and Risk Modelling Approaches for Ensuring Microbial Safety and Quality of Mediterranean Artisanal Fermented Foods” was a research project funded by the EU Program for Research and Innovation Solutions in the Mediterranean Region (PRIMA), which was implemented between 1 June 2019 and 31 May 2023.

In the 2018 PRIMA Call, the ArtiSaneFood project ranked first among all projects within the Topic Food Safety in Local Food Chains; and was the first PRIMA project granted under Portuguese leadership, with a total executed budget of -1.5 million euro.

The project was conceived with the objectives of developing efficient bio-intervention strategies, monitoring and sampling schemes, and an easy-to-use food safety decision support web-based tool for artisanal food producers, aiming to the reduction and control of food-borne pathogens in artisanal fermented foods of meat or dairy origin produced in Mediterranean region. The research workplan was organised in nine work packages, and was successfully implemented by eight partners:

- Instituto Politécnico de Bragança, Portugal
- French Agency for Food, Environmental and Occupational Health & Safety, France
- French Dairy Interbranch Organization, France
- Agricultural University of Athens, Greece
- University of Bologna, Italy
- Ibn Zohr University, Morocco
- University of Cordoba, Spain
- Higher Institute of Biotechnology, University of Manouba, Tunisia

The project’s data and outputs can be continued to be accessed via the links:

Website: <http://www.ipb.pt/artisanefood/>

Repository: <https://zenodo.org/communities/artisanefood/>

Decision-support tool: <https://arti-sane-food-frontend.vercel.app/>

Keynote Lectures

Antibacterial and Antioxidant Activities of Mediterranean Plant and Industrial Coproducts

Nourhène Boudhrioua

Research Laboratory Physiopathology, Alimentation and Biomolecules, LR17ES03, Higher Institute of Biotechnology Sidi Thabet (ISBST) - University of Manouba, BiotechPole Sidi Thabet, 2020, Tunisia.

*Presenting and corresponding author (Oral): nourhene.boudhrioua@isbst.uma.tn

Abstract

Agri-food industries generate high amounts of processing coproducts and are generally disposed of in landfills whereas just a low proportion is recycled into bioactive extracts or biomolecules. Mediterranean spices and herbs are generally included in traditional receipts to develop sensory properties whereas the antibacterial activities of their extracts for potential industrial use were rarely explored. The aim of this work is to summarize and analyse experimental data generated in the framework of the PRIMA-funded ArtiSaneFood project dealing with the potential of industrial vegetable coproducts, some herbs and spice as alternative sources of biomolecules and bioactive extracts to be used as natural antioxidant and antibacterial agents. The DPPH- and ABTS radical scavenging antioxidant activities, the iron chelating activity (ICA) and antibacterial activities against pathogenic bacteria of industrial vegetable coproducts (olives leaves, orange and lemon by-products, date seeds, grape marc extracts) and herbs (mint, fennel seed, tabelle and paprika) extracts were determined. All vegetable extracts were prepared using eco-friendly extraction methods (using water, ethanol, or acetone or a mixture) and freeze-dried extracts. The different extracts were assessed for in vitro antioxidant properties and antimicrobial activities against pathogenic bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 43972, *Pseudomonas aeruginosa* ATCC 9027, *Micrococcus luteus* ATCC 1880, *Bacillus cereus* ATCC 14579 and *Listeria monocytogenes* ATCC 19117). The minimum inhibitory concentration, MIC, varied significantly according to the type of vegetable extract and target pathogenic bacteria. The MIC of investigated herbs and spices varied from 1.56 to 25 mg/L. Herb and spices extracts showed an interesting antimicrobial potential with promising use in meat products biopreservation. Whereas the MIC of the extracts of industrial vegetable co-products showed a huge variation ranging from 1.56 to 200 mg/L. Olive leaves extracts and citrus extracts rich in oleuropein and hesperidin showed the most interesting in vitro antibacterial and antioxidant activities. The effect of these extracts on the main quality attributes of dairy products was examined and showed a general satisfactory overall appreciation with development of distinctive sensory properties in the end products.

Key words: Herb and spices; industrial vegetable co-products; bioactive compounds; pathogenic microorganisms.

Supporting Funds: European Commission: PRIMA-S2-2018- PCI2019-103453 CALL: PRIMA-S2-2018-PCI2019, STICODE, University of Manouba.

Bioprotective Potential of Autochthonous Lactic Acid Bacteria in Moroccan *Jben* Cheese and *Merguez* Sausage

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Abstract

Morocco is known for its popular traditional animal-based foods, including goat's *Jben* cheese and *Merguez* sausage. However, studies have revealed the presence of pathogenic microorganisms, such as *Salmonella* spp. and *Listeria monocytogenes*, in these foods, posing significant public health risks.

As consumers increasingly seek natural, safe, and minimally-processed foods, the interest in natural preservation methods, like Lactic Acid Bacteria (LAB) and their bacteriocins, is growing. This study focused on isolating antagonistic LAB from the indigenous microflora of *Merguez* sausage and goat *Jben* cheese, produced without starter cultures, while also investigating LAB presence in raw materials and other traditional animal-based foods.

In dairy products, 2400 LAB isolates were tested using the agar-spots test, with 292 antagonistic isolates further examined using the agar well-diffusion method. Twenty-three strains demonstrated antimicrobial activity, mainly against *L. monocytogenes*. In the case of *Merguez*, 1440 isolates were screened for antagonistic activity against *L. monocytogenes*, *E. coli*, and *S. aureus*. From a total of 93 isolates with clear inhibition zones, only five showed measurable inhibition against one or more indicator strains. The phenotypic identification tests, coupled with API galleries and 16S rDNA sequence analysis, identified three *Latilactobacillus sakei*, one *Enterococcus faecalis*, and one *Enterococcus durans* strain from *Merguez*. Out of a total of 18 active strains originating from dairy products, 80% belonged to *Enterococcus durans* and *E. faecium*. Fast acid-producing strains Y17 (*E. durans*) and Y252 (*L. sakei*) were applied as starter cultures for fermented sausage, reducing *L. monocytogenes* loads in simulated fresh sausage by 3.6 to nearly 6 log CFU/g. These strains may serve as adjunct cultures to enhance finished product safety.

The findings indicate that autochthonous LAB with antagonistic activity against undesirable microorganisms can effectively improve traditional fermented dairy and meat product safety and quality, offering valuable insights for traditional food producers and consumers.

Key words: Traditional meat products; antagonism; biocontrol; biopreservation.

Supporting Funds: This work was made possible thanks to the PRIMA project ArtiSaneFood (PRIMA/0001/2018) funded by the EU PRIMA program and the Moroccan Ministry of Higher Education, Scientific Research and Innovation (MESRSI).

Exploitation Pathways of the Microbiome Characterisation of Fermented Foods

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Abstract

The microorganisms associated to fermented foods jointly represent their microbiome. Advances in second and third generation sequencing methods, proteomics, metabolomics and computational approaches empowered scientists with the necessary tools to decode the microbial communities circulating in the fermented foods and their metabolic potential.

The exploitation pathways of the microbiome characterisation result in a number of food production applications and innovations. The mapping of the microbiome associated to fermented foods supports the identification of fit for purpose starters and the understanding of the relationships between starters and endogenous microbiome during processing, ripening and shelf life. The clarification of the link between microbiome composition and release of specific volatile metabolites is applied to drive the organoleptic characteristics of fermented foods versus selected aromas.

The composition in genes associated to bacteriocin production and antimicrobial properties is part of the microbiome characterisation and is applied to show the protective effect of specific endogenous microorganisms against the colonisation by foodborne pathogens.

The microbiome of artisanal fermented foods shows differences in genera composition in comparison to industrial fermented foods. Moreover, a huge variability in microbiome composition has been demonstrated in products from different production areas, even within the same country, highlighting the role of the environment in the food microbiome composition.

The presence of foodborne pathogens and their correlation with probiotic bacteria in different types of artisanal soft cheeses produced in the Mediterranean area has been investigated in the ArtiSaneFood project. The results show that the composition in foodborne pathogens clusters with the origin of the artisanal cheese products. The abundance of pathogens negative correlates with that of *Lactococcus* and *Lactobacillus*. On the contrary, the presence of pathogens positively correlates with the presence of virulence factors, as genes coding for motility, and antimicrobial resistance genes.

Key words: Fermented foods; artisanal; microbiome; multi-omics; exploitation pathways.

Supporting Funds: This work was funded within the PRIMA Project ArtiSaneFood “Innovative bio-interventions and risk modelling approaches for ensuring microbial safety and quality of Mediterranean artisanal fermented foods” (PRIMA/0001/2018).

Conduction of Tailored Challenge Testing Experiments in Artisanal Foods: Data Preparation for Modelling Purposes

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Abstract

The design and performance of challenge testing experiments are oriented to the obtaining of high-quality data, what is essential for building accurate predictive models. Challenge tests, defined as studies of the growth or inactivation of microorganism(s) artificially inoculated in food, are normally performed using target strains which are exposed to a range of environmental conditions mimicking real-life scenarios. Artisanal foods normally consists of complex matrices with an heterogeneous mixture of ingredients and elaboration methods which difficult the accuracy of microbial detection and enumeration. Specifically, the indigenous microbiota could affect the behaviour of the target strains so that the applied inoculum preparation, inoculation methods and analytical techniques should be very well standardised in order to provide valid results for models' development.

Data generation for modelling purposes has been dedicated to the estimation of the growth potential and/or calculation of the maximum growth rate, though latest guidelines also describe methods for estimating inactivation parameters. Considerations on how to deal with microbial and batch variability, as well as the data requirements for modelling will be presented.

During the lecture, a description of the most applied protocols for data generation will be given according to the latest ISO/FDIS 20976-1:2018(E) standard for the conduction of challenge tests and the EURL-Lm Technical Guidance Documents for shelf-life estimation of *Listeria monocytogenes* in ready-to-eat foods. The standardisation of methodologies for inoculation and quantification methodologies for data generation would benefit the predictive microbiology community, by increasing transparency and reproducibility.

Key words: Traditional foods; batch variability; inoculation methods; predictive microbiology.

Supporting Funds: The authors are grateful to EU PRIMA program and the International Joint Programming (Project Reference PCI2019-103453) R&D Projects 2019 from the Spanish Ministry of Science and Innovation (Plan Estatal de Investigación Científica y Técnica y de Innovación 2017–2020: State R&D Program Oriented to the Challenges of the Society) for funding the ArtiSaneFood project (PRIMA/0001/2018).

Dynamic Modelling for Ensuring Microbiological Safety of Artisanal Fermented Foods

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Abstract

The fermentation and maturation processes of artisanal foods can be effectively controlled and regulated to provide microbiological stability to the products without the need for heat treatment, but instead based solely on the physicochemical changes during ripening that act as hurdles to undesirable microflora. The objective of this presentation is to show how dynamic predictive microbiology can be applied to characterise the kinetics of foodborne pathogens in artisanal fermented foods. Data obtained from fate studies of *Staphylococcus aureus* in Portuguese raw sausages formulated with 0.0%, 0.5%, 1.0% and 1.5% annatto (*Bixinia orellana* L.) extracts are taken as example.

The dynamic model proposed consists of a log-decay with tail model in differential form as primary model, and a Bigelow model of the D values as a function of pH as secondary model.

$$\frac{dN}{dt} = -kN \left(\frac{1}{1 + C_c} \right) \left(1 - \frac{N_{res}}{N} \right)$$

$$\frac{dN}{dt} = -kC_c$$

$$D = \frac{\ln(10)}{k}$$

$$\log D = \log D_{ref} - \left(\frac{pH - pH_{ref}}{z_{pH}} \right)$$

Such a dynamic model adequately described all survival curves. The addition of annatto extract produced log D* (at reference pH=7.0) slightly higher (1.040 [SE=0.064], 1.040 [SE=0.294] and 1.079 [SE=0.303]) than the control (1.036 [SE=0.142]), whereas z_{pH} tended to be lower with increasing extracts doses (2.426 [SE=0.561], 2.144 [SE=0.230], 2.113 [SE=1.033] and 2.005 (SE=0.904)). Therefore, the addition of annatto extract significantly decreased the time to achieve one log reduction, which in practical terms corresponded to up to 1.04 [SE=0.08] log CFU/g reduction by the end of maturation.

Key words: Log-decay model with tail; Bigelow model; D-value; z_{pH}

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Risk Assessment of Artisanal Food Products: Importance of Monitoring Process Parameters and Microbiological Quality

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Abstract

Surveillance plays a critical role in the assessment of microbial risks by providing valuable data for risk assessors. Contamination levels in raw ingredients, intermediate or final products are commonly used as inputs for quantitative microbial risk assessments. Surveillance is also crucial for food business operators in establishing control measures. To ensure effective control measures, it is necessary to validate them (i.e. establish that they reduce hazards when properly applied), monitor them, and verify that they have been properly implemented. All of these steps require the acquisition of data.

Surveillance activities involve systematic collection, analysis, and interpretation of data on the occurrence, distribution, and characteristics of microbial hazards. Since detection of pathogens can be difficult due to low contamination levels, monitoring can be supplemented by surveillance of process parameters such as pH and temperature.

Food safety metrics such as food safety objectives, performance objectives and process criteria can help establish the link between surveillance conducted by food business operators and risk assessments.

Artisanal products are typically less standardized than other industrial products due to less control of processing conditions and possible heterogeneity of raw materials. This variability can impact the efficacy of monitoring and sampling.

During this presentation, we will showcase examples of calibration of surveillance for controlling risks using both microbiological and process parameters.

Key words: metrics; surveillance; variability.

Supporting Funds: ArtiSaneFood project (grant number: ANR-18-PRIM-0015) which is part of the PRIMA program supported by the European Union. This project aims on microbial safety of artisanal fermented food produced in the Mediterranean region

Session I: Bio-preservatives as agents to ensure quality of artisanal foods

Potential of Essential Oils from Eucalyptus, Peppermint and Pine as Food Preservatives

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Abstract

According to several reports from the World Health Organization (WHO), food spoilage causes various detrimental effects on human health, economy, and on the environment. Subsequently, the demand for food products that adhere to both health and environmental criteria has experienced a surge. This study focused on the use of essential oils (E.Os) as a natural counterpart to chemical food preservatives seen their remarkable bioactive properties, mainly their antimicrobial and antioxidant activities that hinder food spoilage. For this, three aromatic plants known for their national economic and forestry importance were selected to add value to their industrial waste: *Eucalyptus globulus* Labill., *Mentha x piperita* L., *Pinus pinaster* Ait. Their E.Os were extracted using hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD), both optimised with Response Surface Methodology. The antioxidant potential of the three E.Os was evaluated on both chemical and cellular levels. Through the DPPH test, peppermint and pine E.Os extracted using HD showed the lowest EC₅₀ values (8.94±0.76 mg/mL and 11.05±0.63 mg/mL respectively), while through the reducing power test, pine E.O extracted using HD showed the lowest EC₅₀ value of 0.36 ±0.065 mg/mL. On a cellular level, eucalyptus E.O extracted using MAHD and pine E.O extracted using HD inhibited the cellular oxidation by 60 ±7.54% and 59 ±3.65% respectively. The antimicrobial activity was also evaluated, and all of the tested E.Os showed an inhibitory activity against the majority of the 16 tested bacterial strains, with a promising effect on Methicillin-resistant *Staphylococcus aureus*, as well as on the studied fungal strains (*Aspergillus brasiliensis* and *Aspergillus fumigatus*), with MIC and MFC values, mainly of peppermint and eucalyptus E.Os, lower than the positive control (Ketoconazole) for the case of *A. fumigatus*.

Key words: Plant-based; food preservatives; antioxidant; antimicrobial

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Using Byproduct Extracts to Improve the Quality of Traditional Meat and Fish Products

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Abstract

The lipid oxidation is considered as one of the major deteriorating process of meat and fish products quality as well as reducing the shelf life, thereby, leading to off-flavour. Various synthetic antioxidants have been used in food products to prevent lipid oxidation but there is a growing demand for naturally antioxidants. In Tunisia, increasing attention has been given to use industrial byproducts as functional food ingredients since they are rich in beneficial bioactive compounds. Among these types of byproducts, three are three of interest in this work: pomegranate peel, artichoke leaves and distilled myrtles leaves. Two traditional products have been studied; marinated sardine and dried salted sausage. The aim of this work was to investigate the effect of industrial byproducts on the quality and the shelf life of a Tunisian traditional meat and fish products.

Sardines marinated with pomegranate peel and artichoke leaves showed better oxidative stability and higher content of polyunsaturated fatty acids. Higher values of free fatty acids and histamine were found in control samples, whereas adding extracts of pomegranate peel or artichoke leaves significantly decreased total volatile basic nitrogen and trimethylamine during storage. On the other hand, the use of artichoke leave powder when preparing dried sausages (6%) reduced significantly ($p < 0.05$) pH values and lipid oxidation (TBARS). The redness (a^*), yellowness (b^*) and lightness (L^*) values tended to be higher for control than artichoke powder sausages. Addition of artichoke powder decreased ($p < 0.05$) the growth of total viable counts during drying, without any significant effect on lactic bacteria. Finally, the use of distilled myrtles leaves in dried sausages, increased lipid oxidative stability ($P < 0.05$), α -tocopherol content, C18:2-n6 fatty acid and polyunsaturated fatty acids.

Key words: natural antioxidant; marinated sardine; dried sausage; quality.

Evaluation of the Preservative Capacity of *Brassica oleracea* var. *Acephala* Extract in a Bakery Product

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Abstract

Over the years, the consumer’s preference for more natural and healthier products have been noticed. These changes have led the industry and scientific community to focus on finding possible ingredients that can act as substitutes for synthetic preservatives in food products. In this context, the objective of this study was to evaluate the effect of a natural ingredient obtained from *Brassica oleracea* var. *Acephala* biowastes as a substitute for synthetic preservatives in *milk bread*.

For this, a rich phenolic compound extract was obtained by maceration and then lyophilised and incorporated into the food matrix. Physical, nutritional, and microbiological parameters were evaluated at 0 and 3 storage days, and the results were compared with a control *milk bread* (without additives) and a *milk bread* with the synthetic preservative sorbic acid, which is commonly used in the food industry.

The results showed that the addition of the natural ingredient increased the mineral content and had a positive effect in protecting from the growth mesophilic microorganisms in milk bread. However, an adjustment in the concentration of this ingredient is necessary, for better effectiveness in the preserving action, since in previous tests, the extract presented antioxidant and antimicrobial activity of great interest. Even so, it is possible to verify that the *Brassica oleracea* var. *Acephala* extract may be considered as a potential alternative to synthetic preservatives in bakery products.

Key words: Biowaste; phenolic compounds; natural preservative.

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Antioxidant Activity of Garlic Powder During Storage

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Abstract

The use of Spices and Herbs has always played an important part in traditional culinary culture. They have often been used for their medicinal, coloring, and flavoring properties. Recently, scientists have turned their attention to the antioxidant capacities of these bio-ingredients for their potential food bio-preservation capacities providing a powerful alternative for the food industry in response to consumer demand.

Garlic is considered a multi-active spice, it has strong antimicrobial and antioxidant properties due to its rich composition of bioactive compounds, especially phenolic compounds. It is often used in medicinal science and culinary preparations. Nowadays, this plant is incorporated in food products as bio-preservatives.

The aim of this work is to study the antioxidant activity and antimicrobial activity of garlic powder and to introduce it as a bio preservative in an optimized recipe for the seasoning of Tunisian kaddid.

To achieve this objective, the garlic cloves were peeled and cut into thin strips of 1 mm thickness. The samples were then oven dried for 11 hours at a temperature of 60°C. After grinding, the obtained powder (90.7 % of DM and $a_w = 0.37$) was kept in zip bags and kept in dark at room temperature.

The analysis of the antioxidant activity was carried out by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) trapping method with a methanolic extract of garlic powder and showed a color change from dark purple to yellow, indicating antioxidant activity. Garlic powder total polyphenols content for an OD of 760 nm revealed a concentration of 112.633 mg /100 g of powder.

The antibacterial activity of an aqueous extract of garlic powder was determined using the disk diffusion method against seven species of bacteria. *Salmonella arizonae* proved to be the most sensitive to the garlic aqueous extract with a diameter of the inhibition zone of 22 mm.

Keywords: garlic powder; antioxidant; antibacterial activity; polyphenols; bio-preservation.

Extraction Optimisation of Phenolic Compounds through Response Surface Methodology from *Ficus carica* L. Leaves and Bioactive Evaluation

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Abstract

Figs (*Ficus carica* L.) are highly consumed and produced, leading to huge amounts of bio-residues, such as leaves, usually discarded, resulting in significant impacts on environment, society, and economy. Nevertheless, studies have shown that fig leaves are a promising source of compounds with strong bioactivity that can be used as natural food preservatives. In the current work, four varieties of fig leaves, namely Longue d'Aout (La), Bourjassote Noire (Bn), Marseille (Ma) and Pastilière (Pa), were analysed to determine their total phenolics content (TP) by the Folin-Ciocalteu assay. Moreover, in an attempt to optimise the extraction process, the Response Surface Methodology was applied, maximising the yields and purity of the extracts in bioactive compounds obtained by the ultrasound assisted extraction (UAE) technique. External parameters (time, power, and % of solvent) influencing the extraction were studied and the Box Behnken model was applied. Fig leaves were then extracted at their optimal conditions and a mixture of 25% of each leaf extract was evaluated to determine its antioxidant through inhibition peroxidation (TBARS) and cellular antioxidant (CAA) essays; and antimicrobial against food contaminants (microdilution technique) activities. The optimal conditions for UAE ranged between 12-28 min for time, 72-75% for power, and 0-20% for solvent to yield higher amounts of TP (between 2.1 mg/g (Ma) and 7 mg/g (Bn)). The antioxidant activity in the TBARS (EC₅₀ of 120±10 µg/mL) and CAA (65±11%) essays showed promising results. For the antimicrobial activity, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* were the most sensitive bacteria with minimum inhibitory and bactericidal concentrations (MIC and MBC) of 5 mg/mL; and *Aspergillus brasiliensis* was the most inhibited fungi (MIC of 2.5 mg/mL). These results highlight the potential of this bio-residues as an alternative source of compounds with preservative capacity.

Key words: Figs waste; bio-residues; bioactive compounds; ultrasound.

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Stability Studies of Chestnut Flower-Based Ingredient Prospecting Incorporation into Beverages

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Abstract

Industries are turning to agricultural waste by-products from the chestnut (*C. sativa* Mill) production chain as a source of natural additive substitutes. Chestnut flowers and their phenolic-rich extracts have been successfully employed to preserve Portuguese wines, so their potential utilization in other beverage products has been envisioned. Nonetheless, to understand whether natural bioactive ingredients can contribute to greater stability over storage time, it is necessary to evaluate their stability first.

This work aimed at investigating the antioxidant stability of chestnut flower aqueous extract intended to be incorporated into beer and flavoured water under an accelerated stability experiment (60°C for 21 days) and pH variation test (2, 4, 6, 8 and 10) as assessed by DPPH and reducing power (RP) assays. Aliquots of the extract (40g/L) kept in an oven were tested every third day and the DPPH results indicated a progressive rise in the EC₅₀ values between days 0 and 21, with values ranging from 0.15 mg/mL to 1.12 mg/mL. For the RP assay, in which the results varied between 0.18 mg/mL and 2.12 mg/mL over time, the same tendency was noticed. Similar ranges were found in the pH test for each antioxidant assay; in the DPPH test, antioxidant capacity ranged from 0.13 mg/mL to 1.06 mg/mL for pH 4 and 6, respectively, whereas in the RP assay, values ranged from 0.20 mg/mL and 2.54 mg/mL, for the same respective pH 4 and 6. Nonetheless, it is possible to infer from the analysis of the results that the extract antioxidant capacity did not vary significantly face pH changes or over time. It's also noteworthy that the pH test revealed that the best antioxidant potential for either assay was found at pH 4, making this extract ideal for use in beers, which typically have a pH of around 4.

Keywords: natural products; *C. sativa*; antioxidant capacity; thermal stability.

Session: e-Poster flash presentations

Impact of the Incorporation of Thyme and Rosemary Essential Oils on the Physicochemical Characteristics of Fresh Cheese

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Abstract

In order to remedy the problem of microbial contamination, the food industry uses synthetic substances whose function is to preserve the microbiological and organoleptic quality of food products; however, the consumer's tendency is recently oriented towards natural products. In addition to their aromatic value, essential oils extracted from aromatic and medicinal plants have antiseptic and antimicrobial characteristics and can contribute to the preservation of food products. The present study aims to investigate the impact of the incorporation of essential oils of thyme and rosemary on the physicochemical characteristics of fresh cheeses.

A Food Scan device followed the evolution of the physicochemical characteristics relative to the fat, protein and salt content; the analysis was done every 6 days on the fresh cheese samples over a period of 28 days. The sensory analysis was performed towards the end of the shelf life of the flavoured and unflavoured samples (control). The study carried out showed that the samples incorporated with essential oils of thyme and rosemary maintained a stable organoleptic quality on day 21, although with a noted slight increase in pH, which can be explained by the metabolic activity of yeasts and moulds. A light increase in the dry extract of the flavoured cheeses was observed. The other parameters studied (i.e., fat, protein and Salt) were not affected by the incorporation of essential oils.

Key words: Fresh cheese; physicochemical quality; essential oil; sensory analysis.

Phytochemical Content, Antioxidant and Antibacterial Activities of Grape Marc Extracts from Chardonnay and Syrah Varieties Grown in Tunisia

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Abstract

Grape marc is a by-product of the wine industry which represents a rich source of natural bioactive compounds. The present work focuses on the estimation of total phenolics, antioxidant, and antibacterial properties of grape marc extracts (GME) from Chardonnay (White) and Syrah (Red) varieties grown in Tunisia. The total polyphenol content (TPC), total flavonoid content (TFC), tannin content (TC) and radical scavenging activities (DPPH and ABTS) were characterised. The microbicidal effects of both GME against pathogenic microorganisms, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 43972, *Pseudomonas aeruginosa* ATCC 9027, *Micrococcus luteus* ATCC 1880, *Bacillus cereus* ATCC 14579 and *Listeria monocytogenes* ATCC 19117 were determined *in vitro*.

Our results showed the presence of significant difference ($P < 0.05$) between GME from Chardonnay and Syrah cultivars for TFC and DPPH parameters, however no substantial difference ($P > 0.05$) was observed between both marc extracts when considering TPC, TC as well as ABTS antioxidant capacity. In general, Chardonnay GME has relatively higher concentrations of TPC (3.64 ± 0.09 g GAE/100 g d.b), TFC (2.92 ± 0.03 g QE/100 g d.b) and TC (2.01 ± 0.06 g CE/100 g d.b) when compared with Syrah GME (3.45 ± 0.03 g GAE/100 g d.b; 2.19 ± 0.01 g QE/100 g d.b and 1.99 ± 0.07 g CE/100 g d.b, for TPC, TFC and TC, respectively). Nevertheless, Syrah GME has higher DPPH radical scavenging activity (1.00 ± 0.01 g trolox /100 g d.b) than Chardonnay GME (0.92 ± 0.01 g trolox /100 g d.b). Both Chardonnay and Syrah GME showed significant antibacterial activity against Gram-positive bacteria such as *P. aeruginosa*; (MIC = 9.37 mg/mL and MBC > 37.5 mg/mL) and *M. luteus* (MIC = 18.75 mg/mL and MBC > 37.5 mg/mL) but lower activity against Gram-negative bacteria (*S. aureus*, *E. faecalis*, *E. coli*, *S. typhimurium*; MIC and MBC > 37.5 mg/mL). It is worth mentioning that GME from the red cultivar exhibited higher antimicrobial activity than the white one. These results suggest that the GME could potentially be used in food, cosmetic and pharmaceutical industries as bio-preservative agent, or in medicine as new antibiotics.

Key words: Phenolic compounds; antioxidant properties; antibacterial activity; pathogens.

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Antimicrobial Potential of *Eucalyptus* Essential Oils

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Abstract

The effects of chemical food preservatives to human health have become more apparent, and thus, consumers tend to prefer natural analogues. Essential oils (E.Os) and volatiles are posed to be the alternatives to synthetic food preservatives.

In this work, the essential oils of *Eucalyptus* leaves, extracted through hydrodistillation (HD) at a previously defined optimal point (S/L ratio: 48.57 g/L; Particle size: 1 mm; Time: 256.77 min) were tested for their antimicrobial activity, evaluated using the microdilution method, establishing the minimum inhibition concentration (MIC). This analysis was performed on eight different food contamination bacterial strains, as well as two fungal species.

For the Gram-negative bacterial strains, the tested E.Os showed the highest inhibition capacity against *Yersinia enterocolitica* setting the lowest MIC values of all bacteria (0.625% (v/v)). The highest MIC values of 2.5 % (v/v) were sought against *Escherichia coli* and *Salmonella enterica* in exequo. As per the Gram-positive bacterial strains, the tested essential oil exhibited a growth inhibition ability against all of the tested strains, with the lowest MIC value of 0.15 % (v/v) noticed against *Staphylococcus aureus*. However, the highest MIC value of 2.5% (v/v) was found against *Bacillus cereus*.

In terms of the fungi, the lowest MIC value of 0.07 % (v/v) was found for *Aspergillus brasiliensis*, followed by a MIC value of 0.6% (v/v) for *Aspergillus fumigatus*. These results are just slightly higher than the MIC value registered for the positive control, Ketoconazole (0.5 mg/mL).

Overall, eucalyptus E.Os extracted using HD showed a better antifungal activity than antibacterial, with the MIC values being very similar to the positive controls. These findings further increase the pressure for regulatory bodies to allow the use of these molecules as food preservatives, pending the reduction of the potentially imparted odour and smell they can add to food.

Key words: Extraction; volatiles; *Eucalyptus globulus*; antimicrobial; food preservation

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Effect of Different Extraction Methods on Hesperidin Content, and *in vitro* Antioxidant and Antibacterial Activities of Ethanollic Orange By-Products Extracts

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Abstract

The current work aims to examine the effect of conventional solvent extraction (CSE) compared to emerging and innovative extraction methods: accelerated solvent extraction (ASE) and ultrasound-assisted extraction (UAE) when applied with or without a pretreatment process of instant controlled pressure drop (DIC) to intensify extraction, antioxidant, and antibacterial activities. The total phenols, flavonoids and hesperidin contents, *in vitro* DPPH and ABTS radical scavenging assays, iron chelating activity and antibacterial capacity of the extracts were determined for the different extraction methods. The results indicated that DIC pretreatment significantly increased the total phenols, total flavonoids, and hesperidin content. The hesperidin amounts increased by 67% for DIC-CSE, 26% for DIC-UAE, and 141% for DIC-ASE.

The *in vitro* DPPH and ABTS radical scavenging and iron chelating activities of extracts were also significantly enhanced. The antibacterial activities varied according to used extraction methods. The most interesting antibacterial activities for all the tested extracts (CSE, DIC-CSE, UAE, DIC-UAE, ASE and DIC-ASE) were against *S. aureus* ATCC 25923, *Micrococcus luteus* ATCC 1880, *L. monocytogenes* ATCC 19117 and *Pseudomonas aeruginosa* ATCC 9027. All assessed minimal bactericidal activities were superior to 200 mg/mL, regardless of the extraction method used. For DIC-ASE, the minimal bactericidal concentration value was 200 mg/mL against *Enterococcus faecalis* ATCC 29212. Overall, our findings showed that DIC pre-treatment, combined with CSE, UAE, and ASE, appear to be a promising approach for the enhancement of the extraction yield of biomolecules from orange byproducts with the improvement or preservation of *in vitro* antioxidant and antibacterial activities.

Key words: Conventional solvent extraction; accelerated solvent extraction; ultrasound-assisted.

Supporting Funds: European Commission: PRIMA-S2-2018- PCI2019-103453 CALL: PRIMA-S2-2018-PCI2019; MOBIDOC scheme, Promesse project, reference N° 435

Exploring the Effects of Rosemary and Thyme Essential Oils on Merguez Sausage Fermentation: Physicochemical, Microbiological and Sensory Properties

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Abstract

The safety of fresh *Merguez* sausages is of paramount importance in maintaining consumer trust and ensuring a high-quality culinary experience. Implementing effective strategies to enhance the hygienic quality and organoleptic characteristics of these sausages is essential for both public health and consumer satisfaction.

This study investigates the impact of rosemary (*Rosmarinus officinalis*) and thyme (*Thymus satureioides*) essential oils (EOs) on the safety and stability of fresh *Merguez* sausages during fermentation. Antibacterial activities of the EOs were assessed *in vitro* against various pathogens, followed by evaluating the effects of two EO concentrations (1% and 2%) on the sausages' microbiological, physicochemical and chemical properties. A sensory evaluation was also conducted. EOs showed effective inhibition of tested strains, with improved hygienic quality observed in batches containing EOs.

The sausages treated with 1% thyme EO demonstrated the most favourable results in terms of microbiological and sensory outcomes. The combined use of fermentation processes and 1% thyme EO emerges as a promising strategy for enhancing the hygienic quality and organoleptic characteristics of *Merguez* sausage.

Key words: Fresh *Merguez* sausages, Antibacterial, *Rosmarinus officinalis*, *Thymus satureioides*.

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Antibacterial and Antioxidant Activities of Methanolic and Decocted Extracts of *Salicornia arabica*: a Halophyte Plant Growing in Tunis Sebkh

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Abstract

This study was undertaken to assess the radical scavenging activities (RSA), Ferric Reducing Antioxidant Power, FRAP) and antimicrobial activities of Tunisian halophyte *Salicornia arabica* (*S. arabica*). Both methanolic (methanol: 80%, v/v; 24 h, 37°C) and decocted extracts (distilled water, v/v; 20 min, 100°C) were investigated.

The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) were determined using six selected food spoilage and pathogenic bacteria: 3 Gram-positive bacteria (*Bacillus cereus* 193, *Staphylococcus aureus* 4466, *Listeria monocytogenes* 5366) and 3 Gram-negative bacteria (*Salmonella enterica* 4396, *Escherichia coli* 405, *Pseudomonas aeruginosa* 108). The decocted extract showed a FRAP value of 0.56 ± 0.02 g TE/100g extract, ABTS-RSA of 1.73 ± 0.06 g TE/100g extract, and a DPPH-RSA of 1.33 ± 0.01 g TE/100g extract. Interestingly, the macerated methanolic extract of *S. arabica* showed a high FRAP value (4.68 ± 0.15 g TE/100g extract), and radical scavenging activities (ABTS-RSA = 103.07 ± 5.08 g TE/100g extract and DPPH-RSA = 2.93 ± 0.01 g TE/100g extract).

S. arabica methanolic and decocted extracts showed antimicrobial activity against the six reference bacterial strains tested, the MICs and MBCs of these extracts varied from 50 to 150 mg/ml. The decocted extract revealed the best MIC and MBC values against *Salmonella enterica* 4396 (MIC=50 mg/mL; MBC=75 mg/mL); and the methanolic extract revealed the best MIC and MBC values against *Bacillus cereus* 193 (MIC = 100 mg/mL; MBC = 110 mg/mL). These results could open new perspectives to use the decocted extract of *Salicornia arabica* in food biopreservation.

Key words: Antimicrobial; biopreservation; plant-based; natural preservative

Supporting Funds: This research was supported by MOBIDOC scheme, project reference N°417, Session 2019

Phytochemical Contents, Antioxidant and Antibacterial Activities of Date Seeds Extracts from Tunisian Deglet Enour variety

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Abstract

Date (*Phoenix dactylifera* L.) seeds have gained interest as a valuable by-product of the date fruit industry and have been identified as a rich source of functional and bioactive ingredients. In this study, different solvents extraction: water (m/v=1/10, temperature=100 °C; time=15 min), aqueous ethanol 50% (m/v= 1/10, temperature=40 °C; time=3 h), aqueous acetone 50% (m/v=1/10, temperature= 40 °C; time=3 h), and water-acetone-ethanol, (1: 1: 1; V: V: V) (m/v= 1/10, temperature=40 °C; time=3 h) were applied to Tunisian date seeds from *Deglet Enour* variety. The phytochemical contents and antioxidant activity of the extracts were assessed. The antibacterial potency of dried extracts against both Gram-positive (*Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880 and *Listeria monocytogenes* ATCC 19117) and Gram-negative (*Salmonella enterica* ATCC 43972, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027) pathogenic bacteria were determined. The total phenol content of the date seeds extracts varied from 4.47±0.05 (aqueous extract) to 20.86±1.79 g GAE /100 g DM (water-acetone-ethanol extract), the total flavonoids ranged between 11.13±0.36 (aqueous extract) and 17.03±0.15 g QE/100 g DM (water- acetone-ethanol extract). All extracts displayed strong DPPH radical scavenging activity ranging between 2.69±0.00 (aqueous extract) and 3.07±0.01 g TE /100 g DM (aqueous ethanol 50%), and ABTS radical scavenging activity ranging between 12.64±0.30 (aqueous extract) and 12.88±0.45 g TE/100 g DM (aqueous acetone 50% extract). The water-acetone-ethanol extract had the most potent antibacterial effect with MIC values of 12.5 mg/mL (against *Micrococcus luteus*), 25 mg/mL (against *Enterococcus faecalis* and *Escherichia coli*), then the aqueous ethanol 50% extract with 25 mg/mL (against *Salmonella enterica*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*), and then the aqueous extract with 25 mg/mL (against *Staphylococcus aureus* and *Listeria monocytogenes*). The majority of minimal bactericidal concentrations (MBC) values were superior to 50 mg/mL for all the tested extracts.

Key words: Date seeds, polyphenols, antioxidant activity, antibacterial potency.

Supporting Funds: European Commission: PRIMA-S2-2018- PCI2019-103453 CALL: PRIMA-S2-2018-PCI2019; STICODE, University of Manouba.

Phytochemical Content, Antioxidant and Antibacterial Activities of Date Fruit Powder from Deglet Nour Variety

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Abstract

Date fruits are rich in macronutrients and micronutrients with interesting nutritional, technological and biological properties. Deglet Nour is one of the most appreciated fruits due to its distinctive sensory properties. While the nutritional and antioxidant properties of the fresh fruit were largely investigated those of processed date fruit, i.e. fruit powder, were rarely explored. The aim of this work was to assess antioxidants and antibacterial activities of Tunisian Deglet Nour date powder. The total phenol content (TPC), total flavonoid content (TFC), and radical scavenging activities (DPPH and ABTS) of date fruit powder extract (acetone 70%, m/v=1:10, temperature=40°C, time=30 min) were determined. The in vitro microbicidal effects of the extract against both Gram-positive (*Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880 and *Listeria monocytogenes* ATCC 19117) and Gram-negative (*Salmonella enterica* ATCC 43972, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027) pathogenic bacteria were determined. The total phenol content and the total flavonoids were 588.59 mg GAE/100 g DM and 385.39±0.04 mg QE/100 g DM. The date fruit powder extract displayed a DPPH radical scavenging activity of 0.423±0.006 g Trolox/100 g DM and ABTS radical scavenging of 7.16±0.03 g Trolox/100 g. The main phenolic compound determined by LC-MS in dried fruit date extract was quinic acid at 1.58±0.05 (mg/g powder). The minimum inhibitory concentration, MIC, varied between 3.12 mg/mL to 200 mg/mL. The most potent antibacterial effect of date fruit powder extract was noticed against *Micrococcus luteus* (MIC=3.125 mg/mL), *Staphylococcus aureus* and *Listeria monocytogenes* (MIC=25±0.00 mg/mL). All minimal bactericidal concentrations (MBC) values were superior to 200 mg/mL for all the tested extracts and pathogenic bacteria.

Key words: date powder; phenolic compounds; antioxidant properties; antibacterial activity.

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A Meta-Analysis of the *in vitro* Inhibitory Effects of Lactic Acid Bacteria Isolated from Dairy Products against Food-borne Pathogens

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Abstract

Lactic Acid Bacteria (LAB) are a group of microorganisms that are commonly employed in dairy food production, bestowing desirable qualities like texture, aroma, and flavor, and possess bio-preservative features owing to their ability to produce lactic acid and peptides with antimicrobial properties. This review aimed to compile information concerning the *in vitro* antagonistic effect of LABs from dairy origin against selected food-borne pathogens. Electronic search used PubMed, Scopus, and Web of Science databases to retrieve articles published from the year 2012 to 2022. A total of 12 primary studies passed the inclusion criteria and provided observations for *Listeria monocytogenes* (n = 123), *Staphylococcus aureus* (n = 107), and *Salmonella* Typhimurium (n = 45). The main outcome extracted was the mean inhibition zone in mm (ID) and its standard error (SE); and was subjected to univariate meta-analyses using the maximum likelihood method. Results showed that LAB from the *Enterococcus* genus presented the highest antagonistic activity against *L. monocytogenes* (pooled ID = 23.31 mm; SE = 2.59 mm). In addition, *Lacticaseibacillus* had the highest inhibitory effect against *Salmonella* Typhimurium (pooled ID = 14.90 mm; SE = 0.58 mm), while *Lactobacillus* presented the highest effect against *S. aureus* (pooled ID = 15.80 mm; SE = 1.16 mm). Moreover, inocula of *Lactococcus* genus presented the lowest inhibition activity against all three pathogens: *L. monocytogenes* (pooled ID = 11.40 mm; SE = 0.6 mm); *Salmonella* Typhimurium (pooled ID = 6.95 mm; SE = 0.23 mm); and *S. aureus* (pooled ID = 11.44 mm; SE = 0.68 mm). Further insights into specific indigenous dairy LAB with the greatest potential for inhibiting selected pathogens will be obtained through ongoing work.

Keywords: bio-preservation; antimicrobial capacity; inhibition zone; systematic review.

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Biocontrol of *Listeria monocytogenes* CECT 4032 in milk, using bacteriocin-producing strain *Enterococcus mundtii* A2: contamination time effect

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Abstract

The application of lactic acid bacteria in food biopreservation is getting a growing interest all over the world, essentially because of their effectiveness and safety. Based on these criterion, *Enterococcus mundtii* A2, a strain isolated from camel milk was selected for our study. It was applied in UHT whole milk to test its ability to inhibit *Listeria monocytogenes* CECT 4032 serovar 4b growth under different times of contamination. Thus, *E. mundtii* A2 was used as a ferment and a bioprotective culture in UHT whole milk, and *L. monocytogenes* serovar 4b was then added at 0, 12 or 24 h of fermentation. In simultaneous co-culture, the pathogen was reduced by 5.5 log units compared to the control. When *L. monocytogenes* was added at 12 h, it was undetectable after 120 h. Interestingly, the same result was reached within only 6 h when the pathogen was added at 24 h.

These results show the effectiveness of *Enterococcus mundtii* A2; and that such effectiveness was influenced by the time of contamination. In conclusion, the application of this strain to control *L. monocytogenes* in fermented products such as *jben*, cheese and *raib* must be encouraged.

Key words: biopreservation; lactic acid bacteria; *L. monocytogenes* 4b; milk; fermentation.

Microbiological and Genetic Study of *Listeria monocytogenes* Biofilms after their Transfer to Cooked Ham and Simulated *in vitro* Digestion

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Abstract

Listeria monocytogenes is a foodborne pathogen that causes listeriosis disease. Foods implicated in the outbreaks are varied, highlighting fishery products, red meat and ready-to-eat foods such as cooked meat, smoked products or soft cheeses. The transmission of the pathogen is due to the fact that *L. monocytogenes* presents a high ability for survival at refrigeration temperatures and against different stress conditions. In this study, biofilms of three food isolated *L. monocytogenes* strains LI.C5, LI.96 and LI.38 have been created, simulating a transfer process by contact to cooked ham and a posterior *in vitro* digestion carried out at pH = 3, at pH = 2, with and without prior storage under vacuum for 7 days at 7 °C. The results showed that, in both cases, there was growth of *L. monocytogenes* after the digestion process, being lower at pH = 2 than at pH = 3, but constituting in both cases a risk to human health. Specifically, transferred and inoculated cells stored for 7 days at 7 °C reached more than 1 log cfu/g growth increase than those immediately transferred and inoculated. On the other hand, when the digestion was carried out at pH = 2, growth results were quite similar in the case of transferred cells with or without prior storage. However, in the case of the stored and immediate inoculated, there was a difference in growth between 1 and 2 log cfu/g, being higher in the stored ones. Therefore, it can be seen that, during storage, the strains acquired greater resistance to digestion and that cooked ham has a protective effect on *L. monocytogenes*. Finally, a genetic analysis by quantitative PCR will be carried out to confirm the presence of the different virulence genes involved in the different stages studied.

Key words: Foodborne pathogen; contamination niches; digestion; virulence genes.

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Thermal Inactivation Kinetics of *Salmonella* Typhimurium in *Alheira* Sausage Batter

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Abstract

Alheira is a fermented sausage, typically produced in the Northern region of Portugal. These sausages are produced in a very artisanal way, mostly by small and family-run enterprises, which contributes to a product with great variability in its quality characteristics (including physicochemical, nutritional, microbiological, and sensorial). Several studies on the microbiological quality of *alheira* have detected the presence of *Salmonella* spp., which highlights the need to implement a more effective microbiological control during the production of *alheiras*, during the heat treatment phase, considering that *Salmonella* spp. showed survival in the *alheira* during maturation.

The objective of this work was to characterise the heat resistance of *Salmonella* Typhimurium (ST) in *alheira* sausage batter. Two batches of ‘*alheira*’ batter were obtained from a local producer and inoculated with an ST culture to reach $-7 \log$ CFU/g. In bags, 10 g of *alheira* batter was spread and subjected in duplicate to temperatures of 63°, 60°, 57° and 54 °C in an immersion bath, and sampled at 6 time points. A log-linear primary model fitted to each of the inactivation curves estimated the death rates of ST in *alheira* batter at 0.038 (SE=0.008), 0.126 (SE=0.003), 0.273 (SE=0.063) and 2.872 (SE=0.763) log CFU/min at 54°, 57°, 60° and 63°C, respectively, with coefficients of determination ranging from 0.914 to 0.987. Using a Bigelow model, the D value was modelled as a function of temperature, resulting in a log D* of 2.302 (SE=0.304), corresponding to 200 minutes at 50 °C to reduce ST by 1 log; and a z value of 5.016 (SE=0.839) °C. This model allows prediction of D-values and lethality times at other temperatures. For example, to achieve the log 7 reduction lethality of *Salmonella* spp. the centre of the *alheira* must be held for 9.0 seconds at 70 °C.

This study estimated for the first time the thermal kinetic parameters of a traditional Portuguese sausage, and will be useful to producers to define, control and validate thermal processes during the manufacture of *alheira* sausage.

Key words: Raw sausage; D value; z value; log-linear death model; Bigelow.

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Effect of Ebeam Irradiation on the Antioxidant and the Antimicrobial Activities of Defatted Freeze Dried Cow and Camel Milk Fractions

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Abstract

Due to its well-balanced composition of essential elements (proteins, carbs and fats) and its abundance in vitamins and minerals, particularly dietary calcium, milk plays an important role in the daily diet of humans. The most common form of milk consumed worldwide is cow's milk. However, in many dry and semi-arid places, other forms of milk, including that from camels, serve as the primary dairy supply. While milk and dairy products are rarely industrially irradiated, it is nevertheless a safe and ecologically friendly preservation method of liquid and powdered milk. The present work investigated the effect of ebeam irradiation at 5, 10 and 20 kGy on antioxidant and antimicrobial activities of freeze dried cow and camel milk fractions. Milk fraction ethanolic extracts have interesting DPPH radical scavenging activity (values range from $8.71 \pm 0.38\%$ to $32.83 \pm 0.82\%$ for cow milk and from $4.11 \pm 0.86\%$ to $31.44 \pm 0.48\%$ for camel milk) while different fractions have low ferric reducing power ($\leq 2.37 \pm 0.1$ mmol FES /100g powder). The antibacterial potential was evaluated against Gram-negative, Gram-positive bacteria and yeast using the disk diffusion assay. Ebeam irradiation treatment maintains constant the antioxidant activity of all cow milk fractions in term of DPPH scavenging activity, while decrease the DPPH scavenging activity of all the camel milk fraction extracts with the exception of the acid whey camel milk fraction (an increase from $25.94 \pm 1.72\%$ at 0 kGy to $35.68 \pm 1.29\%$ at 20 kGy). The ferric reducing power values of all cow milk fractions were the most resistant to the applied irradiation doses since there was no difference between all the applied doses. In contrast, ebeam treatment promotes (skimmed milk), preserves (sodium caseinate fraction) or decreases (sweet whey and acid whey) the antioxidant activity of the camel milk fraction in term and of ferric reducing power. The zone of inhibition of different milk fractions varied between 0.7 and 1.1 cm and it is generally preserved and or enhanced in irradiated milk fractions.

Key words: Dairy; bioactivity; extracts; liophilisation.

Effect of Herbal Extracts on the Survival of *Staphylococcus aureus* in Goats' Raw Milk Cheese

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Abstract

Previous work from our research group showed that lemon balm, sage and spearmint hydroethanolic extracts possess antimicrobial capacities. Thus, this work aimed to evaluate the antimicrobial effect of these herbal extracts against *S. aureus* in goat's raw milk cheeses during maturation, which have shown moderate prevalence of this pathogen.

Lyophilised herbal extracts were produced using ethanol 70% (v/v) in a shaking water bath. Milk was inoculated with *S. aureus* ($-5 \log$ CFU/g) and 1% (w/w) of each extract was added to the curd, while a non-inoculated control was kept. Maturation occurred at 10°C and 98% RH for 15 days. *S. aureus* counts and pH were determined at specific days. For every treatment, a log-decay function with tail in differential form (with varying D-value), coupled to a Bigelow equation of D-value as a function of pH (with parameters $\log D_{ref}$ at pH 7.0 and z_{pH}) was adjusted.

The models adequately fitted the survival curves, with RMSE of 0.116, 0.063 and 0.057 for spearmint, lemon balm and sage respectively, producing significant parameter estimates ($p < 0.05$). $\log D_{ref}$ was affected by the addition of extracts (spearmint: 0.621 ± 0.061 ; lemon balm: 1.189 ± 0.200 ; sage: 0.996 ± 0.278) in comparison to the controls (0.932 ± 0.166 ; 0.996 ± 0.056 ; 0.796 ± 0.068 , respectively). z_{pH} was higher with the addition of spearmint (3.172 ± 0.660), whereas sage (2.006 ± 0.677) and lemon balm (2.339 ± 0.835), did not affect z_{pH} . Overall, the addition of extracts significantly decreased the time to achieve one log reduction. This work characterises *S. aureus* survival parameters in goat's milk cheeses and demonstrates the utility of these biopreservatives against *S. aureus* during cheese maturation.

Key words: Log-decay model; Bigelow model; D-value; z; dairy

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Exploring the Inhibitory Potential of *Capsicum annum* Extracts Against *Listeria monocytogenes* and Spoilage Bacteria in Fresh Beef Sausages During Storage

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Abstract

Despite being highly nutritious for human consumption, meat and meat products provide an optimal environment for the growth and propagation of pathogenic and spoilage bacteria. The objective of this research was to assess the effectiveness of paprika ethanolic extract in inhibiting *L. monocytogenes* and within a controlled food matrix across varying concentrations.

Fresh sausages made from irradiated ground beef (80% lean), salt, and spices were inoculated with a 2-strain cocktail of *L. monocytogenes* (target, 10^6 CFU/g), individually or in combination, then stored at 4 °C, 8 °C and 12 °C for 5 days. The paprika extracts had strong anti-listerial activity against both strains studied, with a MIC of 3.3 mg/ml and MBC ranging from 3.3 to 13 mg/ml. and showcased inhibitory effect in fresh sausages. To describe the growth/survival of the microorganism in the presence of the extracts, the suitability of three kinetic models were explored. The parameters and the performance of these predictive models was assessed using R software packages. Based on the results, paprika extract was best capable to inhibit the growth of *L. monocytogenes* in a fresh sausage during storage at 4°C and 8°C with a concentration of 6.6% and optimal bacterial reduction was reached at 13.2% of added extract at 3 days for both strains. There was no significant difference in the growth behaviours and the specific growth rates of the two different serotypes of *L. monocytogenes* inoculated.

The findings of this study indicate that these extracts could be utilised to enhance the microbiological safety of food products. Nevertheless, additional research and refinement are necessary.

Key words: Inhibition; *Capsicum* extract; meat product; storage.

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Potential Effects of Syrup and Seeds Powder of Tunisian Pomegranate (*Punica granatum* L.) on Characterisation and Sensory Properties of Camel Milk Yogurt

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Abstract

Camel milk has started gaining interest from scientists and consumers due to its health benefits. The fermentation of camel milk can further increase its nutritional and therapeutic virtues. However, fermented camel milk and especially yogurt is known by a weak texture and poor consumer acceptance. Hence, incorporation of fruits in camel milk formulation leads to health-promoting camel dairy product with an acceptable taste. This study aimed to improve the acceptability and physicochemical qualities of camel milk yogurt by adding pomegranate dried seeds powder and syrup. Three formulations of camel yogurt were prepared with different proportion of pomegranate dried seeds powder (P) and syrup (S). Enumeration F1, F2 and F3 has been assigned to each batch formulation. Formulation F1 contains an equal weight of the two ingredients (P/S=1), F2 is richer in syrup (P/S<1) and F3 is richer in powder (P/S>1). A control formulation T was prepared without the addition of pomegranate derivative for a comparative study. Sensory properties of different formulations were assessed using a 9-point hedonic test. Titrable acidity, dry matter, syneresis, and viscosity of different formulations were examined during 21 days of storage at 4 °C. The results showed that the addition of pomegranate seeds and syrup reduced pH and modified viscosity and chromatic parameters of yogurt samples. The formulation F3 (the richest in powder) has the highest dry matter and mineral content. The formulation F2 (S=P) exuded less water than other formulations. Whereas, incorporation of pomegranate syrup with a high proportion (F3) offers the most desirable formulation (F3) regarding sensory and color evaluation. In conclusion, pomegranate syrup and dried seeds have demonstrated their effectiveness as novel and attractive additives in camel milk processing by serving as natural coloring, sweetening and flavoring compounds.

Key words: New dairy product; formulation; fermentation; quality.

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Genomic Snapshot of Foodborne Pathogens from Artisanal Food Productions of Animal Origin in the Mediterranean Region: Occurrence, Resistome and Virulome

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Abstract

The aim of the present study was to assess the presence and investigate antimicrobial resistance (AMR) and virulence key traits of *Listeria monocytogenes*, *Salmonella enterica* and *Staphylococcus aureus* strains from artisanal fermented meat and dairy productions typically consumed in Portugal, Spain, Italy and Morocco. Over 2,800 samples were collected from raw materials, semi-finished, final products and environmental sites in 4 to 6 batches of each food production between 2019-2021. By implementing a Whole Genome Sequencing (WGS) approach, in silico 7-loci MLST and SNP calling were carried out to assess strains phylogenetic relationships whereas Abricate was used to explore resistome and virulome. Fourty-two isolates were confirmed as *L. monocytogenes* (n=14), *S. enterica* (n=13), and *S. aureus* (n=15). *L. monocytogenes* ST1 and ST8 carried several pathogenic features, such as the full-length *inlA* gene and the *Listeria* Pathogenicity Island 1 (LIP1) and 3 (LIP3). These isolates persisted from the environment to fermented sausage of Spanish artisanal plant. One clone of *S. Paratyphi B* ST43 persisted over one year in the Portuguese meat processing environment to final products and exhibited a high virulence repertoire (i.e., pathogenicity islands SPI, SP2, SP3). Diverse *S. aureus* genotypes were introduced in artisanal meat and cheese facilities of Spain, Italy and Morocco over time as a result of different cross-contamination events, with ST121 harbouring the highest pathogenic potential. Concerning AMR features, many genes encoding for aminoglycosides, beta-lactams, sulphonamides, trimethoprim and tetracyclines resistance were carried by *S. enterica* serovar Hadar and Senftenberg from Moroccan fermented sausages. Despite the overall low prevalence of bacterial foodborne pathogens detected across all productions, enhanced prevention and hygienic control measures are required to strengthening barriers to these biological hazards in the artisanal food chain and thus safeguard consumers health.

Key words: Artisanal foods; Whole Genome Sequencing; virulence; antimicrobial resistance.

Supporting Funds: This work was funded within the PRIMA Project ArtiSaneFood “Innovative bio-interventions and risk modelling approaches for ensuring microbial safety and quality of Mediterranean artisanal fermented foods” (PRIMA/0001/2018).

Metagenomic Investigation of Artisanal Fermented Meat from the Mediterranean Area

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Abstract

The consumption of fermented meat is widespread in the countries of the Mediterranean area. Often these are typical and artisanal high-quality products, in which the fermentation process plays a relevant role in determining the organoleptic characteristics, but, on the other hand, it can represent a source of microbiological hazards due to the absence of standardised production procedures.

In this study, the shotgun metagenomic approach was applied in order to investigate and understand the microbial community structure of different sausages and salami from Italy, Morocco, Portugal and Greece. The DNA of 13 samples (including either technical and biological replicates) were extracted and sequenced by the Illumina platform. The obtained sequences were analysed using MG-RAST to determine the microbial composition, VFDB and RGI to evaluate respectively the presence of virulence factors (VF) and antimicrobial resistant (AMR) genes.

The taxonomic analysis highlighted a strong overlapping between technical replicates and, conversely, high variability between different meats including biological replicates. Several foodborne pathogens were found such as *Salmonella enterica*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* with relative abundance > 5% in at least one sample, together with less represented bacteria including *Yersinia enterocolitica* and *Listeria monocytogenes*. However, the abundance of pathogens did not allow to cluster the samples according to the origin of the meat. Also the analysis of VF and AMR genes indicated an inhomogeneous pattern not linked to the origin neither to the artisanal production process.

In conclusion, our analysis suggested that the artisanal fermented meats from different countries of the Mediterranean area may be vehicles of zoonotic agents and foodborne pathogens. Their taxonomic composition and functional genes are not country-specific and further investigation is in order. From a technical point of view, this study indicates that the shotgun metagenomic analysis workflow is highly reproducible.

Key words: Fermented meat, shotgun metagenomics, foodborne pathogens

Supporting Funds: This study was funded by the PRIMA project ARTISANEFOOD “Innovative Bio-interventions and Risk Modelling Approaches for Ensuring Microbial Safety and Quality of Mediterranean Artisanal Fermented Foods”.

Microbiological Hazards Investigation in an Artisanal Salami Production Plant

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Abstract

In the present study, the occurrence of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli* VTEC was investigated in two batches of artisanal Italian salami tested in winter and summer. Moreover, enumerations of total bacterial count, lactic acid bacteria and *Enterobacteriaceae* were performed as well as monitoring of water activity and pH. Samples were taken from raw materials, production process environment, semi-finished product and finished products.

The results revealed an overall increase of total bacterial count and lactic acid bacteria during the ripening period, along with a decrease of *Enterobacteriaceae*, pH and water activity. No significant difference was observed between the two batches. The enterobacterial load appeared to decrease during the maturation period mainly due to a decrease in pH and water activity below the limits that allow the growth of these bacteria.

E. coli VTEC, *Salmonella* spp. and *L. monocytogenes* were not detected in both winter and summer batches. However, *Klebsiella pneumoniae* was detected in products samples during summer and winter. Except for one isolate, no biological hazards were detected in the finished salami, proving the efficacy of the ripening period in controlling the occurrence of microbiological hazards in ripened salami. Further studies are required to assess the virulence potential of the *Klebsiella pneumoniae* isolates.

Key words: meat product; enumeration; *Enterobacteriaceae*; food-borne pathogens.

Supporting Funds: This work was funded within the PRIMA Project ArtiSaneFood “Innovative bio-interventions and risk modelling approaches for ensuring microbial safety and quality of Mediterranean artisanal fermented foods” (PRIMA/0001/2018).

Microbiological Hazards Investigation in an Artisanal Soft Cheese Production Plant over One Year

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Abstract

The aim of this study was to perform a longitudinal survey in an Italian artisanal factory producing soft cheese to evaluate the inter- and intra-batch variability of physicochemical and microbial parameters on a total of 720 environmental and cheese samples. Cheese samples were additionally collected during 15 days of storage at constant temperatures of 2 and 8°C, as well as a dynamic profile of 2°C for 5 days and 8°C for 10 days. Enterobacteriaceae isolates were identified at species level to have a better knowledge of the environmental and cheese microbiota potentially harbouring human pathogens.

High inter-batch variability was observed for lactic acid bacteria (LAB) and total bacteria count (TBC) in cheese at the end of production but not for pH and water activity. A temperature of 8°C was associated with a significantly higher load of *Enterobacteriaceae* in cheeses belonging to batch 6 at the end of storage, and this temperature also corresponded with the highest increase in LAB and TBC loads over cheese shelf life.

Results from generalised linear mixed models (GLMMs) indicated that drains in the warm room and the packaging area were associated with higher levels of TBC and *Enterobacteriaceae* in cheese. No sample was positive for verotoxigenic *Escherichia coli* (VTEC) or *Listeria monocytogenes*, whereas six *Staphylococcus aureus* and one *Salmonella pullorum* isolates were collected in cheese samples during storage and processing, respectively. Regarding *Enterobacteriaceae*, 166 isolates were identified at species level from all batches, with most isolates belonging to *Klebsiella oxytoca* and *pneumoniae*, *Enterobacter cloacae*, *Hafnia alvei*, and *Citrobacter freundii* evidencing the need to focus on hygienic procedures for cleaning and disinfection especially in warm and maturation rooms.

Further studies should be performed to investigate the potential pathogenicity and antimicrobial resistance of the identified *Enterobacteriaceae* species in artisanal cheeses.

Key words: regional cheeses, linear mixed models; foodborne pathogens.

Supporting Funds: This work was funded within the PRIMA Project ArtiSaneFood “Innovative bio-interventions and risk modelling approaches for ensuring microbial safety and quality of Mediterranean artisanal fermented foods” (PRIMA/0001/2018).

Session 2: Bioprotective lactic acid bacteria in artisanal foods

Assessment of the Bioprotective Capabilities of Lactic Acid Bacteria Isolated from Artisanal Alheira, a Portuguese Fermented Sausage

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Abstract

Alheira is a non-ready-to-eat fermented meat sausage traditional in Portugal, made with cooked poultry/pork meat, bread soaked in the meats' cooking broth and seasonings. The fermentation of this sausage occurs naturally, depending on batter colonization by populations of lactic acid bacteria (LAB) naturally present in the raw materials and in local environments. Aside from fermentation, these bacteria also produce bioactive molecules which aid in the inhibition of pathogens and in development of desirable organoleptic properties in the finished product.

The aim of this study was to isolate LAB present *alheira* sausages and evaluate their potential for application during manufacture as biopreservatives.

Sixty-four *alheiras* from 13 regional producers were analysed and 345 LAB were isolated from MRS and MI7 media and confirmed by Gram staining and catalase test. The antimicrobial activity of the isolates against *Staphylococcus aureus* and *Salmonella* spp. at 37 °C was tested by spot-on-lawn assay. Sixty-three selected strains (43 from MRS, 20 from MI7) were further tested for their antimicrobial activity at 10 °C. Acidification capacity, lactic acid production and proteolytic activity was also determined. The suitability of the strains was evaluated by adjusting two separate Principal Component Analyses (PCA) on MRS and MI7 data.

For MRS strains, PCA explained 73% of data, with PC1 (39%) characterising strains with high acidification, PC2 (20%) was linked to strains with great antimicrobial activity at low temperature and PC3 (14%) allowed the differentiation between isolates with good proteolytic and lactic acid production. For MI7 strains, PC1 (44%) correlated with greater acidification, PC2 (20%) distinguished strains with higher acidification from isolates with good antimicrobial activity against *Salmonella* spp., and PC3 (12%) characterised strains with good inhibition of *S. aureus* at 10 °C. Finally, the PCA allowed the identification of several isolates with both good acidifying capacity and antimicrobial potential, desirable qualities in starter cultures for use in *alheira* production.

Key words: Fermented sausage; biocontrol; pathogens; principal component analysis.

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Biocontrol of *Listeria monocytogenes* in fermented milk by selected *Lacticaseibacillus paracasei* and effect of citrus peel extract on its survival

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Abstract

Listeria monocytogenes is an important foodborne pathogen that has gained relevance and has been identified as a potential contaminant in dairy products during post-processing. The objective of this study was to investigate the effect of citrus peel extract on the viability of *L. monocytogenes* in a fermented milk model prepared using autochthonous strain (*L. paracasei* OWS 23). After milk fermentation (37°C-18 h), two-fold Minimum Inhibitor Concentration of freeze-dried citrus extract was added to the fermented milk samples. They were then individually inoculated with *L. monocytogenes* at -5 log CFU/mL. Microbiological, physicochemical, and antioxidant analyses were performed on samples inoculated or not with *L. monocytogenes* during storage at 4°C for 7 days.

After 2 days of storage a significant decrease ($P < 0.05$) of *L. monocytogenes* was noted in fermented milk with extract compared to non-supplemented samples to reach a 3.76 ± 0.1 log CFU/mL at day 7 (1.97 log CFU/mL decrement from initial concentration). In addition, *L. paracasei* counts increased from 5.92 ± 0.1 log CFU/mL to 6.28 ± 0.2 log CFU/mL during the cold storage. Physicochemical results showed that pH dropped significantly ($P < 0.05$), with lower values obtained for fermented milk supplemented with citrus extract, indicating the mutual effect of *L. paracasei* OWS 23 and citrus extract on the inhibition of *L. monocytogenes* growth during retail storage. Fermented milk supplemented with citrus extract exhibited high radical scavenging activities, RSA (ABTS -RSA = 68.3 % inhibition; DPPH -RSA = 94.1 %). Lactic acid bacteria in synergy with natural extracts enhance bio-preservation of fermented milk.

Key words: Foodborne pathogen; biopreservation; *L. paracasei* OWS 23; plant-based; fate study.

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Meta-Analysis on the Inhibitory Effects of Indigenous Lactic Acid Bacteria Supernatant from Dairy Origin against Foodborne Pathogens

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Abstract

Investigations have been conducted to explore the potential of lactic acid bacteria (LAB) supernatant, obtained by separating bacterial cells from liquid medium through centrifugation, as a natural preservative against foodborne pathogens, given the known antimicrobial properties of LAB. The objective of this systematic review was to evaluate the antimicrobial activity of lactic acid bacteria supernatant against three main microorganisms associated to the contamination of dairy products: *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus*.

Bibliography searches were performed in PubMed, Scopus, and Web of Science databases. A total of 21 primary studies passed the selection criteria and provided 276 observations. The main outcome was the mean inhibition zone diameter in mm (ID) and its standard error (SE). Data was partitioned into smaller segments specific to each pathogen and fitted to obtain meta-analysed values for the inhibition zone, using the maximum likelihood method.

Results showed a total of 82 observations for *L. monocytogenes*, 75 for *S. Typhimurium* and 119 for *S. aureus*. The main genus of LAB obtained was *Lactobacillus* (n=207), although other genus such as *Pediococcus* (n=28), *Enterococcus* (n=21) and *Lactococcus* (n=17) were found. The highest antagonistic activity against *L. monocytogenes* was linked to LABs of the genus *Lactobacillus* (pooled ID = 17.71 mm; SE = 0.88 mm), followed by *Pediococcus* (pooled ID 15.57 mm; SE 0.38mm) and *Enterococcus* (pooled ID 12.72 mm; SE 0.69mm). *Lactococcus*' supernatant displayed the lowest antagonistic effect (pooled ID = 10.34 mm; SE = 1.01 mm). Additionally, *Lactobacillus*' supernatant was observed to be the most effective for inhibition of *S. Typhimurium* (pooled ID 15.98 mm; SE 0.71 mm) and *S. aureus* (pooled ID 16.79 mm; SE 0.91 mm), with *Lactobacillus fermentum* (n=73) and *Lactobacillus plantarum* (n=64) as the most common *Lactobacillus* species used. Finally, the results indicated that *Lactobacillus* supernatant displays strong antimicrobial activity against all three foodborne pathogens analysed. Ongoing work will provide further insight on specific indigenous dairy LAB with the highest potential inhibitory capacity against the selected pathogens.

Key words: antagonism; biocontrol; in vitro antimicrobial capacity; inhibition diameter.

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Exploring the Technological and Safety Properties of Lactic Acid Bacteria Isolated from Artisanal Salchichón: in Search for a Novel Starter

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Abstract

New generational “novel starters” are in artisanal producers’ spotlight for the technological stabilisation of products’ fermentations. These starters are based on autochthonous microbiota adapted to physicochemical product conditions being capable to develop particular sensory properties. Previously to their widespread industrial application, requirements of safety, technological efficiency and economic feasibility must be assessed. A production batch of Spanish dry-cured sausages “salchichón” was microbiologically assessed. A total of 25 colonies grown on MRS medium were picked based on their morphology. Of them, 5 strains lactic-acid bacteria (LAB) were identified by 16S-RNA PCR and subjected to technological evaluation tests, i.e. salt tolerance, exopolysaccharide production, acidification, lipolytic activity; and safety assessment assays, i.e. haemolysis, antimicrobial resistance and *Listeria monocytogenes* antagonistic assays. Monocultures of three *L. monocytogenes* strains isolated from cheese and meat products were used. Quantitative data was statistically analysed from 3 replicates per determination. *Lactobacillus sakei* (4/5) and *Pediococcus acidilactici* (1/5) were identified. All the strains were able to grow in medium containing 10% NaCl and showed similar acidification patterns (from 6.11 to 5.24-5.78 in 9 hours). Only two and three *L. sakei* strains produced exopolysaccharide and haemolysis, respectively, meanwhile none of them showed proteolytic or lipolytic activity. The highest antimicrobial resistance (by disk-diffusion method) was detected against oxacillin (6.00±0.00-11.27±1.13 mm) and vancomycin (6.00±0.00-10.39±1.32 mm). All the *L. sakei* strains showed average values ranging 15.33-19.32mm against *L. monocytogenes* by the spot-on-law technique, meanwhile *P. acidilactici* obtained good results against two *L. monocytogenes* strains (21.78 and 22.39 mm) and failed against the remaining one (3.46 mm). The results showed the potential of some autochthonous LAB strains isolated from artisanal products for their foreseen incorporation as starter cultures. Testing on food matrix must be performed in the near future.

Key words: starter culture; functional products; fermentation process; food safety.

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Effect of Commercial Starter Cultures and Selected Bacteriocinogenic Lactic Acid Bacteria on the Microbiological Quality of Goat *jben* Cheese

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Abstract

Lactic acid bacteria (LAB) are known to produce lactic acid, which reduces the pH of the cheese and inhibits the growth of pathogens. Some LAB strains are capable to produce antimicrobial peptides called bacteriocins that can directly control foodborne pathogens like *Listeria monocytogenes*.

In this study, the effect of commercial starter cultures (CSC) on the microbiological quality of goat *jben* cheese was tested by producing the cheese at a laboratory scale with two CSC "Flora Danica" and "Cheese Culture". The study showed that total aerobic mesophilic bacteria and LAB ranged from 10^6 to 10^9 cfu/g approximately. The decrease in *Staphylococcus* levels to <10 cfu/g may be due to the low pH values. For total coliforms (TC), they disappeared after the third day of storage for the batch made with "Cheese Culture". For the batch made with "Flora Danica", the TC level remained constant during 15 days of storage. For the batch made without the CSC addition, the numbers of total coliforms, staphylococci and yeasts & moulds were higher than the other two batches. Regarding the organoleptic quality of these three productions, it was found that the cheese made with Flora Danica was better than the other two, regarding aroma and flavour, based on colleagues who have tasted it.

The effect of selected bacteriocinogenic LAB on the evolution of *Listeria monocytogenes* in goat *jben* cheese was also assessed. Three batches of *jben* were prepared, one control without LAB, the other batch was produced with *Enterococcus hirae* F420 and the last batch with *Lactococcus lactis* KB2. The inoculation with 10^4 cfu/g of a cocktail of three strains of *L. monocytogenes* CECT 4032, CECT 7467 and CECT 935 was made just before storage. The study showed that the *Lactococcus lactis* strain inhibited the growth of *L. monocytogenes* during 18 days of storage.

Key words: Moroccan cheese; fate study; *Lactococcus lactis*.

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Study of the Bioprotective Potential of Three Different Lactic Acid Bacteria Cocktails against *L. monocytogenes* in Vacuum-Packaged Cold Smoked Rainbow Trout

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Abstract

The implementation of bio-preservation treatments in smoked fish products can be a promising strategy through the use of lactic acid bacteria (LAB) with bio-protective potential. The objective of this study was to evaluate the inhibitory capacity of different LAB cocktails against *L. monocytogenes* in vacuum-packed smoked rainbow trout fillets; and to characterise the interaction between the different LAB vs *L. monocytogenes*. For this, a three-strain cocktail of *L. monocytogenes* isolated from fishery products was inoculated with different combinations of LAB strains; *Leuconostoc carnosum* + *Lactococcus lactis* (commercial L1 starter), two strains of *Lactobacillus pentosus* I19+ *L. pentosus* LPG1 (L2 LAB isolated from table olives) and *L. pentosus* LAB2 + *Lactiplantibacillus plantarum* LP15 (L3 LAB isolated from table olives), using an inoculation ratio of 2:3. Two different conditions were assessed; one batch stored at a constant temperature of 5 °C for 21 days and another batch under dynamic temperature conditions, simulating thermal abuse due to failures in the cold chain. The results obtained at 5 °C were satisfactory since out of the three LAB cocktails used in the test two of them achieved an inhibition of the growth of *L. monocytogenes*. Starting from the initial load of the pathogen (4 log CFU/g) and after 21 days storage with the L1 LAB cocktail, the final concentration of the pathogen was reduced in 0.5 log CFU/g. Using the L3 LAB cocktail, the *L. monocytogenes* concentration remained constant over storage ranging within ± 1 log CFU/g from the initial load. At dynamic storage temperature, the L1 cocktail was the most effective reducing the concentration of *L. monocytogenes* in more than 0.5 log CFU/g throughout the shelf-life. It is concluded that the L1 LAB cocktail is the one with the greatest spectrum of inhibition against *L. monocytogenes* since it was able to reduce the initial load, both under static and dynamic conditions. The L3 cocktail from LAB strains isolated from table olives was the best of the two LAB cocktails with the same origin, because it presented a bacteriostatic effect against the pathogen, thus being a valid bioprotective culture for RTE fishery products.

Key words: Biopreservation; biocontrol; challenge testing; RTE fish.

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Impact of Commercial Starter Cultures on the Microbiological and Physicochemical Properties of Traditional Merguez Sausages

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Abstract

Traditional *Merguez* sausages are fresh meat products that are popular in Morocco. However, traditional production methods can lead to inconsistencies in quality and safety. In this study, we investigated the effect of commercial starter cultures on the microbiological and physicochemical properties of *Merguez* sausages during fermentation and ripening.

Three batches of sausages were produced using different starter cultures, including a control batch with spontaneous fermentation, a batch (SP) with *Staphylococcus xylosum* and *Pediococcus acidilactici*, and a batch (LS) with *Lactobacillus sakei* and *Staphylococcus carnosus*. The microbial counts, physicochemical characteristics, and chemical composition of the sausages were measured throughout an 18-day processing period.

Our results showed that the use of starter cultures significantly reduced *Enterobacteriaceae* counts in the sausages, with the LS batch showing the greatest reduction of approximately 2 log CFU/g compared to the control and SP batches. In addition, the LS batch had lower pH values than the other batches, indicating a more acidic environment that could prevent the growth of harmful bacteria. Although the use of starter cultures did not significantly affect the chemical composition of the sausages, the SP batch had a higher fat content than the other batches. In conclusion, our study demonstrates that the use of commercial starter cultures can enhance the safety and quality of traditional *Merguez* sausages. Specifically, the use of LS starter culture led to a significant reduction in *Enterobacteriaceae* counts, which are indicators of potential foodborne pathogens. The use of starter cultures can be an effective way to improve the safety and quality of fermented meat products, and our findings could be useful for the meat industry and consumers as well.

Key words: Fermented sausages; lactic acid bacteria; biocontrol; food safety.

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Effect of the Drying Process and Incorporation of Natural Preservatives in the Viability of Lactic Acid Bacteria in Yogurts

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Abstract

Dairy products are considered one of the most consumed foods in the world, and yogurts are highly demanded for their flavour and nutritional properties, also including macronutrients, vitamins, minerals, or probiotics offered. To avoid food waste, alternatives to preserve food are highlighted, such as the addition of food preservatives or the production of powdered foods. Therefore, the objective of this work was to evaluate the survival of total lactic acid bacteria when natural extracts (rosemary, basil, and sage) were incorporated into yogurts, as well as to investigate their viability after the freeze-drying process. Yogurts were prepared in the laboratory, and lyophilised plant extracts (30 mg/kg) were added. Total lactic acid bacteria were analysed for 14 days, in addition to evaluating the lactic acid and lactose contents by HPLC-DAD and HPLC-RI, respectively. Subsequently, the viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were evaluated in freeze-dried yogurts without the addition of vegetable extracts. Regarding the results obtained, there was a slight increase in the lactic acid bacteria counts, in addition to an increase in lactic acid and a reduction in the lactose content over 14 days, confirming the yogurt fermentation process. For the viability of *S. thermophilus* and *L. bulgaricus* during the lyophilisation process, a slight reduction of both bacteria was observed, but within the limits established by the Codex Standards (minimum of 10^7 CFU/g). Therefore, the incorporation of plant extracts and the freeze-drying process did not significantly change the yogurts regarding the viability of the lactic acid bacteria, remaining a probiotic food.

Key words: Fermented milk; powdered foods; lyophilisation; natural products.

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Session 3: The microbiome of fermented foods

Genomic and Phenotypic Analysis of Lactic Acid Bacteria Isolated from *Alheira*, a Portuguese Traditional Fermented Sausage

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Abstract

Lactic Acid Bacteria (LAB) play a crucial role in determining the flavour, texture, and nutritional properties of fermented foods. This study aimed to investigate the diversity of LAB in *alheira*, a traditional Portuguese fermented meat sausage. For this, 63 LAB isolates were obtained from 28 *alheira* samples collected from different regions of Portugal, including Vimioso, Mirandela, Vinhais, Mogadouro, Pova de Lila, Bragança, and Valpaços. The LAB isolates were reactivated in Man, Rogosa, and Sharpe or M17 agar and then subjected to molecular identification using the 16S ribosomal gene. Genomic DNA extraction was carried out with the GF-1 Bacterial DNA Extraction Kit, and amplification of the 16S gene was performed by 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-CTA CGG CTA CCT TGT TAC GA-3') primers. The sequencing reactions were made with BigDye™ Terminator v3.1, and samples were purified with SAM/BigDyeX Terminator™ bead solution. Capillary electrophoresis was conducted with the SeqStudio Genetic Analyzer. The BLAST algorithm was used to obtain alignments with at least 97% identity with reference sequences from the NCBI database. In addition to molecular identification, the LAB isolates were also evaluated for *in-vitro* proteolytic activity (mm), L-lactic acid (g/L), acidifying capacity, and antimicrobial capacity against *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Staphylococcus aureus*. The data obtained were assessed by Principal Component Analysis (PCA). Genetic analysis of samples revealed a diverse LAB population, *Enterococcus* was the most abundant genus (31.7%), followed by *Leuconostoc* (19%) and *Lactilactobacillus* (17.5%). The acidification was mainly contributed by *Enterococcus* (*E. faecium* and *E. durans*), while *Leuconostoc*, *Pediococcus*, and *Lactiplantibacillus* exhibited higher inhibition diameters values against *S. Typhimurium* (PC1=58%, PC2=19.3%), *L. monocytogenes* (PC1=53.9%, PC2=19.4%), and *S. aureus* (PC1=56.3%, PC2=18.5%) in addition to high proteolytic activity, as revealed by the PCA analysis. Finally, the results indicated that *Lactiplantibacillus plantarum* was the LAB species with higher inhibitory effects against the three pathogens analysed. These results indicate that a comprehensive characterisation of LAB can be valuable for improving food safety and quality control.

Key words: non-RTE sausages; food biotechnology; molecular identification.

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Integration of Contributing Factors Affecting Microbiological Diversity and Food Safety throughout the Artisanal Salchichón Manufacturing

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Abstract

Salchichón is a Spanish dry-cured fermented sausage especially linked to the artisanal production. General low pH/a_w, high NaCl and nitrate/nitrite presence has led to considered them microbiologically safe ready-to-eat products. This work was aimed at the microbiological assessment of two salchichón artisanal manufacturers (A/B). A total of 171 samples were collected from meat batter, food-contact surfaces (FCS), finished products (different ripened) and air among 3 different production batches. Microbiological analysis of aerobic mesophilic bacteria (AMB), lactic-acid bacteria (LAB), coagulase-positive *Staphylococcus* spp. (CPS), total coliforms, *Enterobacteriaceae*, *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes* and *S. aureus* were performed. Presumptive-pathogen isolates were preliminary identified by MALDI-TOF and confirmed via 16S rRNA PCR/PFGE. Overall, meat batter AMB counts (4.20-6.82 log cfu/g) was positively correlated with CPS in A where a 33.33% of meat samples tested positive to *S. aureus*. Meat batter AMB in B was correlated with *Enterobacteriaceae* and tested positive to *L. monocytogenes* (20%), revealing hygiene deficiencies. FCS results highlighted in B, where the highest contamination loads were found in the filling hopper (3.97 log cfu/cm²) and mincing hopper (3.83 log cfu/cm²), being *L. monocytogenes* also detected (5.56%). Regarding finished salchichón samples, 16.67% (5/30) and 10% (3/30) tested positive for *L. monocytogenes* and *S. aureus*, respectively. Two of those positive samples (21 days ripened) presented physicochemical values allowing *L. monocytogenes* growth (pH 5.93, a_w 0.93). The PFGE pathogens analysis revealed different contamination sources. These results benefit the artisanal producers for a better understanding of the potential role of good manufacturing practices for their products quality recognition.

Key words: dry-cured fermented sausages, cross-contamination; *Listeria monocytogenes*; public health.

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Phylogenetic Analysis of Lactic Acid Bacteria species from Cheese: a Comparison between Taxonomy and Physicochemical Characteristics

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Abstract

Lactic acid bacteria (LAB) have a wide genetic potential and are applied in areas such as food production, intestinal health, and genetic engineering. Bioinformatics holds fundamental tools for the interpretation of genomic data, allowing for the understanding of DNA and its sequences. Thus, the objective of this work was to evaluate the phylogenetic relationship between 40 LABs from goat's raw milk cheese, using the genetic sequences of the 16S ribosomal gene and physicochemical characteristics (i.e., pH, [lactic acid] and antimicrobial activity against selected foodborne pathogens). Sequences of LAB identified as *Loigolactobacillus coryniformis* (2.5%), *Leuconostoc mesenteroides* (25%), *Lactococcus lactis* (25%), *Lactococcus cremoris* (10%), *Lactiplantibacillus plantarum* (2.5%), *Lacticaseibacillus paracasei* (20%), and *Enterococcus faecalis* (15%), were analysed in the R software, where a multiple sequence alignment was performed with the MSA package. Then, an identity matrix containing the squared root of the pairwise distances was calculated with the Seqinr package, which allowed for the identification of 3 main clusters, formed by the species *Leuconostoc mesenteroides* (s=12%), *Lactococcus lactis* (s=28%), and *Lacticaseibacillus paracasei* (s=26%). Moreover, a phylogenetic tree was built with the Neighbor-Joining method ($R^2=0.98$), which revealed moderate variability among sequences of the same species from the positions assumed in the cladogram. Additional descriptive statistics allowed the comparison between phylogenetic and taxonomic data. Results showed that LAB species *Lactiplantibacillus plantarum* presented greater lactic acid production power (54.8 mg/100gDM) and inhibitory capacity against *Listeria monocytogenes* (mean inhibition halo at 10°C, 16.76mm and at 37°C, 9.33mm), *Staphylococcus aureus* (mean inhibition halo at 10°C, 14.46mm and at 37°C, 7.88mm), and *Salmonella spp.* (mean inhibition halo at 10°C, 10.63mm and at 37°C, 12.14mm). Finally, *L. plantarum* has been shown to present a diverse functional genome, which may account for its greater potential compared to all species analysed. Further research will yield more understanding of LAB's diverse metabolic and physiological characteristics.

Key words: cheese microbiota; taxonomy; phylogenetic tree; antagonism.

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Safety Evaluation of Lactic Acid Bacteria and its Bacteriocin Production Based on Whole Genome Sequencing

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Abstract

Lactic acid bacteria (LAB) have been widely used in the production of fermented foods. Safety concerns have arisen to potential effects of bacteria, such as virulence and antimicrobial resistance transfer. The aim of this study was to assess the safety of 10 LAB isolates based on complete genome sequences. The complete genome of *E. faecalis* OWS78, *L. paracasei* OWS 23, *L. fermentum* OWS 107, *E. durans* OWS 153, *L. fermentum* OWS 33, *L. fermentum* OWS 96, *E. faecium* OWS 116, *E. faecium* OWS 64 and *L. fermentum* OWS 120 were sequenced on Illumina MiSeq Platform. Putative virulence factors, antimicrobial resistance genes, and amino acid decarboxylase production were predicted using the virulence factor, and UniProt databases. LAB strains were also examined for bacteriocins by comparing their sequences to similar sequences in the UniProt database. The 10 LAB strains were examined for 18 genes related to virulence factors. *E. faecalis* OWS78 was found to contain genes for gelatinase (gelE), collagen adhesin (ace), enterococcal surface protein (esp), and serine protease (sprE). The detection of hyaluronidase (hyl) genes in *E. faecium* OWS 38, OWS 116, and OWS 64 makes it inappropriate to use these isolates in food products. The absence of surface virulence factors, such as cytolysin, hyaluronidase, gelatinase, amino acid decarboxylase genes, and antibiotic resistance genes, especially aminoglycoside phosphotransferase, is a positive trait to consider for *L. paracasei* OWS 23, *L. fermentum* OWS107, *L. fermentum* OWS96, *L. fermentum* OWS33, and *L. fermentum* OWS120. Genes responsible for the production of bacteriocins were found only in *L. paracasei* OWS23 for LSEI 2386 and for bacteriocin immunity proteins. Results showed that out of all the strains, *L. paracasei* OWS23 and *L. fermentum* OWS107, OWS96, OWS33, and OWS120 are the most promising candidates for application in food technology and food biopreservation.

Key words: WGS; virulence factors; antimicrobial resistance; bacteriocins.

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Biofilm-forming Ability of *Listeria monocytogenes* and *Staphylococcus aureus* strains isolated from Artisanal Fermented Products Processing

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Abstract

Cross-contamination represents a relevant cause of *Listeria monocytogenes* (LM) and *Staphylococcus aureus* (SA) presence in ready-to-eat products. Biofilm-forming ability results crucial for their ubiquity and persistence in food processing environments, representing an important economic impact for producers. Variable factors affecting microbial dynamics in artisanal fermented products manufacturing are closely linked to biofilm-forming strains harbouring. The *in vitro* evaluation of the biofilm-forming potential of LM and SA strains isolated from meat and dairy artisanal industries was aimed. Out of 24 strains, five SA and three LM were isolated from meat samples, three SA and five LM from dry-cured fermented sausages, two SA from raw milk cured cheeses, two SA from raw goat milk and four LM from meat industries surfaces. Inocula were activated in Tryptone Soya Broth (TSB), adjusted to 0.5 McFarland concentration and subsequently serial-fold diluted to 4 log cfu/mL. From this, 2 mL were added to a 24-well plate where also 1 cm² steel coupons were placed. After 3 days incubated (37 °C), formed biofilms were extracted; firstly by carefully PBS washings (planktonic cells removal) and later, erosively with peptone water (adhered biofilm detaching). Plating and incubation was carried out following the LM and SA enumeration ISO standards. All the isolates tested (100%) showed high biofilm-forming ability. Enumeration representing SA biofilm formation obtained an average of 7.93±0.80 log cfu/mL (maximum c.a. of 8.68 log cfu/mL) with not significant differences (P>0.05) between the different types of samples and isolates. A slightly lower average count was obtained for LM, with 7.34±0.48 log cfu/mL (maximum c.a. of 8.83 log cfu/mL), also without significant differences (P>0.05) obtained between different samples and strains. These results confirmed the biofilm-forming ability of strains isolated from fermented food processing, reinforcing the importance of their study for foodborne outbreak prevention. Molecular and genetic studies should be performed to provide further information about biofilm formation mechanisms, adhesion genes and improved control strategies.

Key words: cross-contamination; ready-to-eat products; stainless steel; food safety.

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Session 4: Predictive modelling in artisanal foods

Estimation of Growth Parameters of *Listeria monocytogenes* under Different Conditions of Mexican Oregano Essential Oil (*Poliomintha longiflora* Gray), pH and NaCl

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Abstract

Food loss and diseases caused by the prevalence of foodborne pathogens and their resistance to preservation methods in meat products have great relevance for food safety. *Listeria monocytogenes* has developed the ability to adapt to stressful conditions in meat like addition of salt and chemical preservatives. Natural additives are an alternative to reduce the use of chemical additives and could improve the product's organoleptic quality without affecting microbial safety. Oregano essential oil has been considered as an antimicrobial agent related to its high concentration in oxygenated monoterpenes like carvacrol and thymol and its status as GRAS additive allows its use in meat products to reduce the growth of pathogens and to improve production processes.

In this study, a cocktail of *L. monocytogenes* was previously adapted to salt and sodium nitrate concentrations typically found in fermented meat products. Then, the cocktail was inoculated in Brain Heart Infusion broth with modified conditions of NaOH (0 and 3.5%), pH (5.5 and 6.5) and Mexican oregano (*Poliomintha longiflora*) essential oil: pure and fractioned, at the following concentrations: 0, 0.05, 0.01 and 1% (v/v). Growth of the cocktail of *L. monocytogenes* was measured using absorbance techniques in a Bioscreen C and growth rate and detection times were estimated as microbial kinetic parameters. Inhibition of *P. longiflora* essential oil against *L. monocytogenes* was achieved at the highest concentration of fractioned oil (1%) but at 0.05%, it was observed a synergistic activity with pH and salt. Results showed that the use of fractioned oil in low concentrations can be efficient since a reduction in growth rate up to 86.18% was shown. Synergistic activity with fractioned oil and other preservation methods like application of mild temperatures and packaging systems against *Listeria* and other foodborne pathogens should be analysed.

Key words: Absorbance methods; food preservation; growth rate; natural preservatives.

Supporting Funds: This project is funding by Consejo Nacional de Ciencia y Tecnología (CONACyT) with the scholarship number 1184786.

Assessing the Efficacy of Bioactive Mint Extract in Controlling *Listeria monocytogenes* Growth during Fermentation and Drying of Traditional Dried Sausages: a Mathematical Modelling Approach

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Abstract

The development and application of predictive models are evolving to evaluate the growth and no-growth boundaries of *L. monocytogenes*, a foodborne pathogen often associated with meat products prepared under a variety of adverse conditions and innovative biocontrol alternatives.

The aim of this work was to conduct an experimental design to mathematically assess the behaviour of *L. monocytogenes* in dried, spontaneously fermented sausages supplemented by three different concentrations of mint extract (*Mentha spicata*). The inoculation of one strain of the pathogen was performed considering a scenario of a highest contamination level under environmental conditions of manufacturing found in small scale Tunisian manufacturer. The interaction between the mint extracts and *L. monocytogenes* was monitored during fermentation (10±1 °C, 48h) and drying (27 ±1°C, 18 days) while analysing LAB counts, water activity and pH. Different approaches were adopted to model the interaction between the levels of the extract and the pathogens using R software; an approach using Huang model and omnibus model to describe growth phase and the decay model to describe the pathogen inactivation. Results showed that the greatest beneficial effect of the added extract was in inhibiting the development of *L. monocytogenes* during the drying phase for all treatments even though the aw was determinant for starting the microbial inactivation in the fitted model. After the sixth day of drying, *L. monocytogenes* counts were reduced by 0.68 log CFU/g, 0.99 log CFU/g and 1.95 log CFU/g for all treatments, respectively. The growth potential of the pathogen was strongly affected with increasing levels of mint extract in both growth and inactivation phase. pH and LAB counts did not present significant differences amongst treatments ($p > 0.05$).

To conclude, the mint extract is an effective bioprotectant against *L. monocytogenes* in traditional dried sausages and the fitted interaction models was able to satisfactorily predict the inhibitory effects.

Key words: Meat product; bioactive extract; *Listeria monocytogenes*; microbial kinetics.

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Quantifying and Modelling the Bioprotective Effect of *Latilactobacillus sakei* AE127, *Lactococcus lactis* AE89, and *Enterococcus mundtii* AE24 Against *Listeria monocytogenes* 7467 on Vacuum-Packed Cold-Smoked Moroccan Trout

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Abstract

The present research evaluated the potential of three strains, namely *Latilactobacillus sakei* AE127, *Lactococcus lactis* AE89, and *Enterococcus mundtii* AE24, which were isolated from fish products in Morocco, as bioprotective cultures against *Listeria monocytogenes* 7467 (CECT) in cold-smoked farmed Moroccan trout at 12°C. The strains exhibited the ability to control the growth of *L. monocytogenes* within six days of storage and lower the pH of the medium.

To explain the inhibitory effects of selected lactic acid bacteria on *L. monocytogenes*, a microbial competitive interaction model based on the Lotka-Volterra approach was evaluated. The proposed bio protection strategy included inoculating the product at a ratio of 1-2 (pathogen: bioprotective, log CFU/g). With constant storage at 12 °C, the kinetic growth parameters of both microorganisms in monocultures and the inhibitory functional parameters of the tested interaction models in cocultures were estimated.

The results demonstrate the efficacy of the strains studied in controlling pathogen growth on fish products; and the proposed modelling approach aims to reduce the risk of listeriosis associated with the consumption of fish products. It may also support the establishment of a bioprotective culture-based strategy for smoked trout.

Key words: Fish product; lactic acid bacteria; microbial competition.

Omnibus Modelling to Describe the Effects of Thermisation on *Staphylococcus aureus* in Goat’s Raw Milk

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Abstract

Milk thermisation has been proposed as a strategy to improve the safety of cheeses made from unpasteurised milk as it reduces bacterial counts while preserving the organoleptic characteristics associated with raw milk cheeses. Hence, the aim of this work was to characterise the heat resistance of *S. aureus* (SA) in goat’s raw milk subjected to thermisation using omnibus modelling (simultaneous fitting of primary and secondary models). Five mL of milk were placed in sample bags and inoculated with SA (7 log CFU/mL).

The inactivation experiments were carried out in a water bath at 55, 58, 61, 62.5 and 64 °C, and the sampling was performed at appropriate times. After removing the samples from the water bath, they were immersed into an ice bath. Upon cooling, SA determination was performed. SA concentration measured at time *i* when subjected to condition *j* was estimated as:

$$Y_{ij} = Y_{0j} - \left(\frac{t}{\chi_j}\right)^{\beta_j} + \varepsilon_{ij} \quad Y_{0j} = Y_{0\text{mean}} + u_j$$

$$\sqrt{\chi_j} = a_1 + a_2 * \text{Temperature} + a_3 * \text{Temperature}^2 + v_j$$

$$\sqrt{\beta_j} = b_1 + b_2 * \text{Temperature} + b_3 * \text{Temperature}^2$$

The omnibus approach allowed to described well all the inactivation curves and estimate SA inactivation parameters in heat-treated milk. The positive intercepts a_1 (343.6±14.87) and b_1 (74.32±9.293) allude to the concavity of the fitting and the quadratic effect of temperature on $\sqrt{\chi}$ and $\sqrt{\beta}$. The negative linear effects of temperature on $\sqrt{\chi}$ and $\sqrt{\beta}$ ($a_2 = -10.87$; $b_2 = -2.427$) were anticipated since higher temperatures should lead to shorter inactivation times. The correlation coefficient between a_1 and b_1 was 0.779.

Validation of the model at 58 °C, 61 °C and 62.5 °C by the “leave-one-out method” was successfully performed, demonstrating its aptitude to be used in the design of lethality treatments to achieve specific reductions of SA in goats’ raw milk, thus contributing to the enhancement of the microbiological quality and safety of raw milk cheeses.

Key words: Weibull; cheesemaking; milk treatment.

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Oregano Essential Oil and Processing Conditions: A Predictive Model for Effective *Salmonella* spp. Inactivation in Sausage Medium

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Abstract

Salmonella-related foodborne illnesses, particularly from contaminated meat products, remain a substantial public health concern. This study aimed to develop a predictive model for *Salmonella* spp. inactivation in a simulated sausage medium (SSM) by examining the effects of oregano essential oil (OEO), pH, temperature, and water activity (a_w).

A Doehlert matrix design was employed, incorporating OEO (0 to 0.3%, v/w) into SSM, adjusting pH levels from 4.7 to 6.8, varying final a_w between 0.88 and 0.93, inoculating with a *Salmonella* spp. cocktail, and storing at temperatures from 8 to 20°C. Weibull models were used to fit the generated survival curves under these varying conditions. A response surface (RS) model, based on a quadratic polynomial equation, was employed to model the inactivation curves under the combined effects of OEO, pH, temperature and a_w , yielding two inactivation parameters (δ [h] and p).

The RS model accurately predicted *Salmonella* inactivation, as demonstrated by the RMSE and %SEP values obtained for δ and p . RMSE and %SEP values for the RS model were 0.14 and 13.32% for δ , and 0.09 and 8.60% for p , respectively. Additionally, the study highlighted the importance of meat product formulation on *Salmonella* inactivation induced by essential oils, alongside the recognised effects of plant extracts. The developed RS model effectively predicts *Salmonella* inactivation in SSM based on OEO, pH, temperature and a_w ; emphasising the need to consider meat product formulations' influence on *Salmonella* inactivation induced by essential oils.

This research can contribute to the development of efficient control measures for preventing *Salmonella* spp. transmission in the food industry.

Key words: Survival; Weibull model; response surface; Doehlert matrix; meat products.

Supporting Funds: The authors are grateful to the EU PRIMA program and the Moroccan Ministry of Higher Education, Scientific Research and Innovation (MESRSI) for funding the ArtiSaneFood project (PRIMA/0001/2018).

Microbiological and Physicochemical Quality Categorisation of Artisanally Produced *Alheira* Fermented Sausages in Northern Portugal

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Alheira is a fermented sausage, non-ready-to-eat, traditionally produced in Northern Portugal. Due to its artisanal production, *alheira* presents high variability in its nutritional, physicochemical, and microbiological composition. Thus, the aim of this study was to characterise the microbiological and physicochemical properties of *alheira* sausages produced by different regional producers; and to evaluate the associations between relevant attributes. Finished products from 16 factories were analysed, for a total of 80 samples. Mesophiles, lactic acid bacteria (LAB on MRS and MI7 agar), *Salmonella* spp., *Staphylococcus aureus* and presumptive *Clostridium perfringens* counts, as well as physicochemical properties (pH, water activity (aw), moisture, ash, protein and fat) were determined. Principal Component Analysis (PCA) of these variables was performed to construct quality maps. Three significant components were extracted, accounting for 64.7% of data variability. PC1 (29.3% of the data), which is highly correlated with ash, moisture, carbohydrates and protein, and highly but inversely correlated with fat, appears to characterise nutritionally richer sausages. PC2 (18.7%) is strongly correlated with LAB, and negatively with pH, apparently characterising the extent of fermentation. Finally, PC3 (16.7%) is strongly associated with pH and *S. aureus* and more moderately with *C. perfringens*, which likely represents the development of pathogens due to weaker/slower fermentation. The projection of samples in three-dimensional space also allowed the differentiation of three clusters of factories, i.e., three types of sausages: Cluster 1, comprised of 8 factories, presented average values of pH and protein, and lower values of fat, LAB and *S. aureus*, the latter possibly indicating less manipulated sausages during manufacture; Cluster 2, with 3 factories, presented sausages with lower pH values, protein, and *S. aureus* counts, and higher values of fat and LAB; and Cluster 3 (5 factories), with simultaneously high values of LAB, *S. aureus* and pH, indicating higher manipulation of *alheira* during manufacture and ineffective fermentation.

Key words: Raw sausage; pathogens; pH; food safety; cluster analysis; factorial analysis.

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Assessing the Impact of Starter Cultures on the Behavior of *Salmonella* spp. and *Listeria monocytogenes* in Dry-Cured Fermented Sausages

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Abstract

Listeria monocytogenes and *Salmonella* spp. have been identified as some of the most frequent causes of food safety alerts linked to the consumption of dry-cured fermented sausages in Spain (latest on 2021). A wide variety of contamination sources are described, making important the understanding of their behaviour throughout all the production chain. The ripening process plays a particular role, where the antimicrobial effects of lactic-acid bacteria (LAB) result fundamental. The aim of this study was to assess the influence both of natural LAB (nLAB) and added starter cultures (scLAB) on the behaviour of *L. monocytogenes* and *Salmonella* spp. during Spanish *salchichón* ripening. Lab-scale *salchichón* prototypes (80 g) were manufactured with formulated meat batter stuffed into permeable plastic bags by vacuum-packaging (30-35mm Ø). These were separately inoculated with a three-strain cocktail of each pathogen (ca. $\approx 6-7$ log cfu/g), previously temperature-adapted (12 °C for 5 d) grown in modified TSB containing salt (25 g/kg) and sodium nitrite (0.15 g/kg). During samples ripening (15 d, 15 °C), physicochemical parameters (pH, a_w) and microbial populations (LAB and pathogens) were monitored. *Salmonella* spp. showed a mean population increase of 1.05 log cfu/g in nLAB samples (maximum ca. 8.25 log cfu/g), but growth was inhibited in scLAB samples. Growth ability of *L. monocytogenes* was shown both in nLAB and scLAB samples, showing differences in the growth potential (1.96 and 1.70, respectively), lag phases (36.32 and 150.02 h, respectively) and maximum growth rates (0.008±0.003 and 0.011±0.007 log cfu/g/h, respectively). Homogeneous LAB populations were obtained at the end of the ripening stage (8.71-9.18 log cfu/g), regardless of the sample type. Similarly, final a_w values were around 0.960, meanwhile pH slightly increased from 5.22 to 5.84. These results contributes to food safety improvement during *salchichón* manufacturing; although a better understanding of fermentation dynamics and the biopreservation potential should be further studied.

Key words: artisanal products; biopreservation; fermentation process; food safety.

Supporting Funds: EU PRIMA program and the International Joint Programming (Project Reference PCI2019-103453) R&D Projects 2019, in the frame of the Plan Estatal de Investigación Científica y Técnica y de Innovación 2017–2020: State R&D Program Oriented to the Challenges of the Society, for funding the ArtiSaneFood project (PRIMA/0001/2018). Also, Ministry of Science and Innovation of the Spanish Government for the research project PID2019-108420RB-C31-ASEQURA.

Comparative Study of Hybrid Optimisation Methods in the Extraction of Antioxidant Phytochemicals from Grape Seed (*Vitis vinifera*)

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Abstract

This work seeks to value waste from the wine industry in Portugal by recovering bioactive compounds from grapes by modelling and optimizing the total phenolic content and antioxidant activity of grape seed extracts by the integrative use of Response Surface Methodology (RSM) and Artificial Neural Network coupled with Genetic Algorithm (ANN-GA), in addition to investigating the correlation between the content of phenolic compounds and the potential for free radical scavenging. The hydroethanolic extracts of grape seed were obtained by microwave-assisted extraction using a three-level three-factor experimental design applied to RSM and ANN-GA, to optimise the extraction parameters and verify the influence of independent variables of the compound recovery process. The two dependent variables considered in this study were the content of total phenolic compounds, determined using the Folin-Ciocalteu method, and the free radical scavenging potential of 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The results revealed that the ANN-GA provided better predictability and greater precision than the RSM algorithm. The extracts obtained by microwave-assisted extraction provided better results in terms of antioxidant activity and phenolic compound content compared to the other technique. Furthermore, the results suggest that the potential to eliminate DPPH free radicals may be directly related to the content of phenolic compounds in the extracts, as the values for each response variable are equivalent in all extracts. Future studies will be carried out to evaluate the bioactive potential of grape seeds in other aspects and their possible incorporation into food matrices as a preservative agent.

Key words: Bioactivity; experimental design; modelling; waste valorisation.

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Effect of Mexican Oregano Essential Oil (*Poliomintha longiflora* Gray) on the Thermal Inactivation of *Listeria monocytogenes*: An In vitro essay

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Abstract

Listeria monocytogenes is a foodborne pathogen known for its growth and survival ability at the wide spectrum of temperatures used in the processing and preservation of food. Meat products could be a potentially hazardous foods that supports the growth of *L. monocytogenes*, therefore the implementation of thermal processes requires correct control of time-temperature. Moreover, thermal inactivation models are a valuable tool used in microbiological risk control to allow food safety. The D-value is a parameter that indicates the required time to achieve the reduction of one logarithm of the microbial population at a constant temperature. The inhibitory capacity of natural antimicrobials such as carvacrol and thymol have been evaluated to reduce D-values using mild temperatures, being oregano essential oil considered one of the most representative in Mexico due to its wide use in several artisanal recipes.

The aim of this study was to evaluate the effect of Mexican oregano (*Poliomintha longiflora*) pure (PO) and fractionated (FIV) essential oil on the thermal inactivation of *L. monocytogenes* in a meat simulated medium (MSM), taking into account a control treatment (without essential oil). The essay consisted of the inoculation of a known concentration of the inoculum on the MSM previously added with essential oil at a sublethal concentration (0.06% v/v), then the treatments were placed in a thermostatic bath at constant temperature and surviving cell counts were performed at appropriate intervals between 0-30 min. Primary Weibull models have been fitted to observed data to obtain microbial inactivation parameters (δ and p values). D-values (minutes) observed (RMSE<0.30) at 52°C were 8.66±1.51, 0.95±0.43 and 3.56±1.01 and at 54°C were 1.81±0.51, 0.89±0.24 and 1.92±0.42 for Control, IV and OP, respectively. Results showed that FIV can improve thermal processes at mild temperatures. Further studies will be achieved to validate these results in a meat product.

Key words: Natural antimicrobials; D value; food preservation; listeriosis.

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Session 5: Miscellaneous topics on quality of traditional foods

Bio4Drinks Project: the Obtention of Natural Multifunctional Ingredients for the Beverage Industry

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Abstract

Traditionally the beverage industry uses synthetic additives to preserve, intensify or improve/modify the final products physical-chemical, biological or sensorial characteristics. However, synthetic additives like sulphites, mainly used as preservatives added to alcoholic and non-alcoholic beverages to prevent oxidation and bacterial growth, have been associated with harmful side effects for consumers like allergic reactions and/or asthma, due to their toxicity. Thus, there is a growing demand for new atoxic additives, mainly derived from natural sources, and with a multifunctional character linked with antimicrobial and antioxidant activities, and enzyme inhibitor capacity. This multifunctional character is an interesting approach when considering the development of innovative flavoured waters, by combining hydration with the antioxidant potential resulting from the extracts. In addition, the multifunctionality is also important regarding the preservation of beverages like craft beer, which is often faced with the loss of beverage quality due to the appearance of undesirable flavours and aromas (off-flavours) and modifications of the physical properties during storage. These are associated with the exposure to environmental factors such as light, oxygen and temperature, which impart the oxidation of some beer constituents. In this regard, the Bio4Drinks project is devoted to the obtention of natural multifunctional ingredients for the beverage industry, to promote the innovation both in the natural extracts and beverage industries, through the research and development of multifunctional natural ingredients. In this context, the project was focused on the research of natural bioactive compounds from the chestnut and wine sectors by-products, with enhanced bioactivities and suitable properties to be used as beverage bio-preservatives and functional additives. This strategy is ultimately contributing to enrich the natural extract market and increasing the sustainability of the beverage industry within the circular economy context.

Key words: Beverage preservation; flavored water; craft beer; multifunctional extracts.

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Evaluation of the Influence of Intrinsic Properties on the Microbiological Quality of Traditional Portuguese *Transmontano* Hard Cheese made from Raw Goat’s Milk

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Abstract

Transmontano goat’s cheese is a traditional Portuguese cheese with Protected Designation of Origin (PDO) certification, made with raw milk from the *Serrana* goat, an autochthonous breed from Northern Portugal. The cheese-making is based on traditional methods with little modification over the years, and the final product is a matured, extra hard cheese with a slightly spicy taste. Because of its artisanal production, the quality attributes of *Transmontano* cheese can be quite variable.

The aim of this study was to evaluate the variability of selected quality properties between cheese batches, as well as the development of hygiene/safety indicator microorganisms (total viable counts [TVC, a.k.a. mesophiles], lactic acid bacteria [LAB], *Clostridium* spp., *Listeria* spp. and *Staphylococcus aureus*) in cheese during ripening, and how it is affected by milk/cheese’s intrinsic properties (i.e. pH, water activity [a_w], fat, protein and ashes content, and lactic acid concentration).

Raw milk and cheese during ripening were sampled from four production batches surveyed from an artisanal producer. Mixed models showed that the ripening process substantially reduced the variability of the physicochemical and microbiological qualities between batches of cheese.

The development of mesophiles in cheese was mainly affected by milk’s pH, a_w and initial TVC load as well as by initial pH and a_w of cheese, and to a lesser extent by protein and fat contents; meanwhile, the growth of LAB was mainly determined by milk’s pH, a_w , and initial LAB counts. The continuing drop of *Clostridium* spp. during ripening was regulated by all the intrinsic properties mentioned above, whilst the inactivation of *Listeria* spp. was highly associated to pH (from milk and from cheese at production day) and lactic acid concentration. *Escherichia coli* O157:H7 and *Listeria monocytogenes* were not detected in any of the analysed samples.

Key words: Ripened cheese; lactic acid bacteria; *Staphylococcus aureus*; *Clostridium* spp.; *Listeria* spp.

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Effect of Chestnut Flower Extract on the Flavour Stability of Craft Beers

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Abstract

Microbreweries have seized their market share by introducing artisanal beverages that possess unique sensory attributes. Craft beers are brewed in small batches, allowing for flexibility in ingredient selection and the production of diverse styles of beer, the quality of which is largely related to its flavour. However, these products are susceptible to a number of oxidative reactions that negatively impact their original flavour and are responsible for their short shelf lives. Whereas some synthetic additives have been used to limit beer oxidation, efforts have been directed to find natural alternative ingredients that can enhance product quality while ensuring safety. The objective of this work was to examine whether a polyphenol-rich ingredient from *Castanea sativa* flowers may stabilize the flavour of craft beers over storage time. Spray-dried aqueous extracts of male chestnut flowers from the Bragança region were incorporated into Indian Pale Ale (IPA) craft beers produced by a local microbrewery. The extract incorporation was carried out either before bottling or in two separate additions before the dry-hopping and the bottling. Volatile organic compound analysis by HS-SPME-GC-MS and sensorial analysis by a trained panel were performed monthly over the course of storage (4 months). Volatile profile results suggested not only the lack of negative interference and as well as a stabilizing effect of the chestnut flower extract in the beer flavour, with no compounds related to beer off-flavours being detected. Findings from the sensory evaluation reported that the initial aroma of incorporated beers differed slightly from the control, however, no oxidation principles were detected in beers added by natural extract, in contrast to the control. In summary, the chestnut flower extract was suitable to enhance flavour stability and is a promising source of natural antioxidants that may extend the shelf-life of craft beers.

Keywords: beer quality; phenolic compounds; beer flavour stability; antioxidant; beer shelf-life.

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Physicochemical properties and biological activities of fermented camel milk: A comparative study with fermented cow milk

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Abstract

Besides biopreservation, fermentation could enhance the nutritional and functional properties of milk. Commercially available starter cultures are from bovine-based dairy industry being not competitive with endogenous microbiota of camel milk, which might not give a desirable final product. This work investigated the physicochemical, antioxidant and antihypertensive properties of fermented camel milk, by an autochthonous probiotic strain, in comparison with fermented cow milk.

Camel and cow milks were fermented by the probiotic strain *Lactococcus lactis* LEFS29, previously isolated from spontaneously fermented camel milk. Both unfermented and fermented camel and cow milks were assessed for their physicochemical properties (pH, acidity, viscosity, color, protein, lactose, ash and organic acids). The *in vitro* antioxidant activity was evaluated using two tests including DPPH radical scavenging activity and ferrous ion chelating activity. The antihypertensive activity was evaluated *in vitro* using the angiotensin-converting enzyme (ACE) inhibitory assay.

The results showed that the fermentation of camel and cow milks by the autochthonous strain *Lactococcus lactis* LEFS29 increased their acidity, viscosity as well as their color parameters (L^* , a^* and b^*). The chemical composition of fermented milks was similar to that of unfermented milks except for the lactose content, which decreased after fermentation. The level of lactic, acetic and propionic acid increased after fermentation. Lactic acid was found to be the major organic acid in fermented milks. Interestingly, the fermentation process increased the antioxidant and antihypertensive activity of both types of milk. Fermented camel milk possessed higher DPPH radical scavenging activity (64.25 %), ferrous ion chelating activity (71.09 %) and antihypertensive activity (77.20 %) compared to fermented cow milk (all the activities < 54%).

Our findings suggest that fermented camel milk using the autochthonous strain *Lactococcus lactis* LEFS29 could be considered as a functional food.

Key words: camel milk; fermentation; probiotic; antihypertensive.

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Magnesium and Manganese Induced Changes in Chemical, Nutritional, Antioxidant and Antimicrobial Properties of Pansy and Viola Edible Flowers

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Abstract

Edible flowers of pansy and viola were hydroponically grown with increased levels of Mg (+Mg), Mn (+Mn), a combination of both Mg and Mn (+Mg+Mn), or reduced levels of their combination (namely the control treatment; C). Nutritional profile, free sugars, tocopherols, and phenolic compounds were determined. The results indicated that Mg enrichment of nutrient solution resulted in a decrease in plant growth and mineral accumulation in flowers; however, it improved photosynthetic performance by increasing chlorophyll content and accumulation of certain minerals. A total of 12 total phenolic compounds were tentatively identified for viola and pansy. In viola, non-anthocyanin flavonoids, namely quercetin-3-O-rutinoside, showed higher amounts in both treatments (-Mg-Mg, +Mg, +Mn and +Mg+Mn), with +Mg (32.777 ± 0.652 mg/g dw) being higher. As for the total anthocyanin profile, delphinidin-3-(4"-p-coumaroyl)-rutinoside-5-glycoside was high in all treatments, especially in +Mg (6.314 ± 0.461 mg/g dw). In pansy, the major non-anthocyanic flavonoid was quercetin-3-O-rutinoside for all the treatments, being higher in +Mg (26.401 ± 0.954 mg/g dw). For the tocopherol profile, alpha tocopherol was identified, standing out in the control treatment (11.01 ± 0.04 mg/100g fw) in the case of viola and in +Mg for pansy plants (11.8 ± 0.1 mg/100g fw). These samples showed antioxidant potential for the TBARs assay, especially in viola plants treated with +Mg (81 ± 1 µg/mL) and in pansy for the combined +Mg+Mn treatment (93 ± 3 µg/mL). As for the cytotoxic profile, in the case of viola the +Mg+Mn treatment showed the highest potential against the NCI-H460 cell line (56 ± 4 µg/mL), whereas in pansy, the control treatment was more efficient (50 ± 3 µg/mL). Considering the anti-inflammatory profile, only viola showed anti-inflammatory activity in both +Mg and +Mn treatments (346 ± 2 and 319 ± 27 µg/mL, respectively). The edible flowers exhibited antimicrobial potential, especially against the strains of the strains of *Bacillus cereus*, *Salmonella Typhimurium* and *Yersinia enterocolitica*. Pansy and viola edible flowers are rich in functional, nutritional, and bioactive compounds, making them interesting candidates for application in the food industry as functional agents.

Keywords: Hydroponics; Nutritional profile, bioactive compounds, edible flowers.

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Anti-Tumoral Properties of *Allium roseum* on Breast Cancer Cells

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Abstract

Allium roseum (AR) is a species of wild garlic that has been traditionally used in medicine. Researchers have looked into the usage of AR as an anti-cancer and food preservative. The use of AR extracts and compounds as a food preservative has been demonstrated to be effective in preventing the growth of foodborne pathogens and delaying lipid oxidation. This helps to retain the quality and safety of food products. The possible anti-tumoral effects of AR compounds in cancer have been the subjects of recent research. However, it has been widely demonstrated that Mediterranean diet is enriched with plant-based nutrition, is associated with a lower risk of chronic diseases in the prevention of cancer. AR contains a range of beneficial plant compounds that are responsible for most of health benefits. The AR main phenolic compounds include flavonoids and phenolic acids that are essential for health benefit with its biological activities including anti-inflammatory, antioxidant, and anticancer properties. Current study indicates that AR extract exert a remarkable effect by modulating cancer cell death and proliferation on Breast Cancer (BC) cell lines. Further studies on animal clinical trials are essential for identifying the AR as a candidate for its beneficial effects on BC treatment and help to formulate its products as an integral part of the dietary and pharmacological intervention of patients with BC. The study contributes also to the discovery of pharmacological responses by identifying the affected genes and their functions.

Key words: Mediterranean diet; pharmacological intervention; anticancer properties.

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