

INNOVATION DRIVEN AGRIFOOD BUSINESS

Abstracts Book

2 ABRIL 2025

Centro de Congressos
Super Bock Arena
Porto-Portugal

Dear Colleagues,

It was with great pleasure that we welcomed you to the third edition of Dare2Change® conference, held again in the beautiful city of Porto.

This international scientific conference, co-organized by Colab4Food, INIAV I.P. and PortugalFoods, aims to bridge the gap between academia and industry within the agri-food sector.

Great challenges such as the pandemic, war, demographic pressure, and climatic changes, have highlighted the urgent need for innovative food technologies/solutions and foods ingredients that meet the demand, respecting global sustainability goals. This includes the production of more nutritious and safer food, reducing waste based on reuse and circular economy principles, producing new food products/ingredients using less (or alternative) natural resources, optimizing technological processes to ensure food quality and extend shelf life. While there is certainly a long way to go, the excellence of Portuguese academia in this area, coupled with the strong collaboration with the food industry, will boost the development of effective solutions and their fast introduction on the market, benefiting the Portuguese industry on the international stage. The exceptional quality of the Portuguese products is not new, but challenges are titanic and required the most seamless coordination ever. This conference expects to make room for this collaboration to arise, while addressing pressing topics.

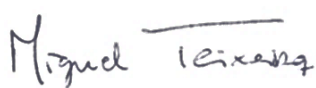
The scientific committee created a program with sessions on pressing/emerging topics, where participants had the opportunity to discuss their latest findings in many areas of agri-food sector, encouraging networking opportunities. This year selected topics include: Food Development and Production; Nutrition, Health, and Well-being; Sustainability and Circular Economy; Digitization and AI in the Agri-food Sector.

This Book of Abstracts, contains 192 abstracts, presented as posters at the 2025 edition of Dare2Change®, that answer to different hurdles within the four main topics of the conference. This figure reflects the great dynamic and diverse nature of the Portuguese agri-food ecosystem.

We sincerely appreciate your presence at Dare2Change®, which greatly enriched the conference and played a pivotal role in its success. We also thank the sponsors for their commitment and support, essential to guarantee the success of Dare2Change®.

We are looking forward to welcoming you again in the next edition of Dare2Change®!

The Organizing Committee,



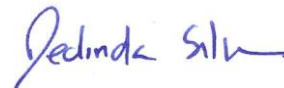
MIGUEL TEIXEIRA

Scientific & Executive Director
(Colab4Food)



CARINA ALMEIDA

Principal Researcher
(INIAV)



DEOLINDA SILVA

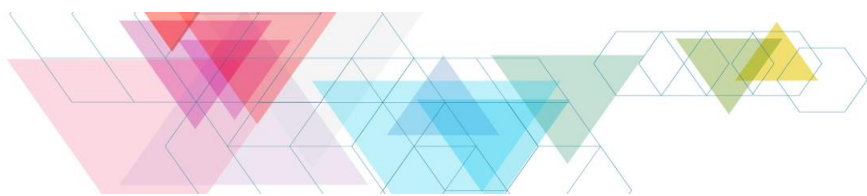
Executive Director
(PortugalFoods)

INDEX

ORGANIZING COMMITTEE	14
OPERATIONAL TEAM	14
SCIENTIFIC COMMITTEE	14
ADVISORY COMMITTEE	14
PROGRAM	15
ABSTRACTS OF POSTER PRESENTATIONS	16
 DIGITALIZATION AND AI IN THE AGRI-FOOD SECTOR	 17
▪ Building a community of practice to CATALYSE food safety breakthroughs.....	18
▪ High-quality high-impact augmented virtuality system for the evaluation of the impact of context on consumer perception and hedonics	19
▪ Gamified online training to improve motivation and knowledge retention in food industry training.....	20
 FOOD DEVELOPMENT AND PRODUCTION	 21
▪ Developing plant-based protein formulations as alternatives to egg white protein in vegan ham-like products	22
▪ 3D Printing vegan snack: A sustainable innovation in food production	23
▪ Impact of thermal treatment on <i>Talaromyces trachyspermus</i> spores inactivation ..	24
▪ Killer yeasts as sustainable solutions for foodborne pathogens control.....	25
▪ Innovative approaches toward natural, healthier, and clean-label meat products ..	26
▪ Innovation on vegan ham analogues using new protein sources.....	27
▪ Evaluation of the technological and microbiological characteristics of cooked smoked bacon with nitrite reduction.....	29
▪ How to communicate with adopters of insect-based foods: insights from a cross-cultural study in Portugal and the UK	30
▪ Development of Innovative Ready To Use Industrial Pastry Preparations Using Insect Based Ingredients	31
▪ Colour improvement of halophyte plants for application as salt substitutes in ham products.....	32
▪ Optimizing the extraction of elderberry anthocyanins for application as natural colorant in ham formulations	33
▪ Hyperbaric Inactivation – A novel nonthermal methodology to destroy <i>Bacillus subtilis</i> spores at room temperature?	34
▪ Exploring the antibacterial potential of cell-free supernatants from probiotic strains against foodborne pathogens.....	35

- Hyperbaric storage as a preservation methodology to control the germination and development of *Clostridium perfringens* spores..... 36
- Emulsion-filled gels for 3D printing of customized foods 37
- Functionalized Plant Proteins as Stabilizers for 3D-Printable Pickering Emulsions 38
- Hyperbaric storage does not substantially impair functional properties of egg yolk while assures microbiological safety 39
- Microencapsulation of a green microalga (*Chlorella vulgaris*) for its valuation as a feasible ingredient in pear snacks 40
- Ensuring Authenticity: Advanced qPCR Solutions for *Tenebrio molitor* and *Hermetia illucens* in Feed and Food Products 41
- Willingness to Try Insect-Based Foods in Portugal and Mexico: A Cross-Cultural Consumer Study 42
- Impact of Photosensitive Nets on Phenolic Compound Content in Redlum Apples 43
- Around the World in 51 Octopus Dishes..... 44
- Effect of cherry and quince juice on the second fermentation of kombucha 45
- Innovative Valorization of Cookie By-Products for Sustainable Production of Prebiotic Fructo-Oligosaccharides 46
- A full insight into the valorization of wheat by-products 47
- Designing a healthy canned meal: chub mackerel (*Scomber colias*) and vegetables 48
- Vegan cheeses - product development and physical- chemistry characteristics.... 49
- Olive Leaf Extract – loaded hydroxyapatite Pickering emulsions: A novel approach to mayonnaise-like food sauces 50
- Cowpea as substitute to soybean in the fermentation of miso paste: Nutritional and sensory evaluation of two varieties: 'Cara Verde' and 'Arroz' 51
- A question of generation? – A comparative study of microbiota composition in subsequent generations of SCOBY and kombucha..... 52
- Unravel the impact of emerging parasites in wild fish and their risk in the Agrifood Chain 53
- Reimagining Waste: Exploring the Nutritional and Biological Power of a Freeze-Dried Snack based on Broccoli Stalk Powder 54
- Sensory Characterization of *Hericium erinaceus*: A Lexicon-Based Approach 55
- Exploring nutritional enhancement of a ready-to-eat spinach-mash with algae 56
- Quantifying adulteration in game meat products: Is it possible? 57
- Ultra-high-temperature ohmic heating of fruit pulps..... 58
- Valorisation of post-harvest *Solanum melongena* plant biomass as a source of bioactive ingredients for yogurt fortification 59
- Vibrational spectroscopy applied to distinguish between Honey PDO Regions 60
- Technological strategies to increase soluble dietary fibre in cloudy apple juice and effect on oligo- and polysaccharides 61

- Development of a new Beetroot Snack – Impact of Pulsed Electric Fields (PEF) pretreatment on the Physicochemical and Bioactive Properties..... 62
- Evaluation of antimicrobial efficacy of current methods to control fire blight (*Erwinia amylovora*) 63
- Oleogels and their different applications: Ham analogues and chocolate spreads 64
- Nutritional and antioxidant properties of clean-label yogurt added fermented fruit preparations..... 65
- Formulation and characterization of two clean-label, low-fat butter alternatives 66
- Factorial Design Study of Pulsed Electric Fields Parameters to Improve the Rehydration Ratio of Freeze-Dried Banana Slices 67
- Moderate pressure pasteurization combined with oxygen scavenger: an innovative approach to improve physical properties of salmon..... 68
- Black soldier fly larvae oil: A game-changer in enhancing fillet quality of high-value mediterranean fish 69
- Advancing algae-based foods: Bridging academia and industry for culinary innovation 70
- Encapsulated aromas for enhancing canned tuna flavour and reducing salt 71
- C-Phycocyanin as a natural alternative to the synthetic blue food dye E133 in beverages..... 72
- Exploring the use of oleogels as alternatives to palm oil in chocolate spread formulations 73
- Sodium reduction in canned tuna: effective strategies for a healthier product..... 74
- Development of protein bars with insect flour (*Tenebrio molitor*): Microbiological, nutritional and sensory evaluation..... 75
- Transforming orange and banana peels fermented with *Yarrowia lipolytica* into nutritious snack bars 76
- Sodium selenate vs sodium selenite: Which is the best choice for selenium biofortification of Swiss chard microgreens? 77
- Potentially toxic element contamination of European City Soils and Implications for Urban Farming Food Safety 78
- Protein fractions from seaweeds: evaluating fractionation techniques to improve nutritional quality and efficient extraction..... 79
- Improving the nutritional quality of pea and soybean microgreens with phenolic-enriched nutrient solutions 80
- Assessment of histamine and biogenic amine variability in fish and fishery products: implications for food safety..... 81
- Impact of dry fractionation on the distribution of faba beans' and peas' antinutrients 82
- Fermentation as an approach to improve the nutritional value of grass pea-based food products 83



▪ Determinant physico-chemical properties to assess technological performance of organic flours	84
▪ Efficient and scalable spray-drying of encapsulated oleoresins for sustainable ingredient production.....	85
▪ Incorporation of <i>Tenebrio molitor</i> flour in olive pâtés and almond butters: A sustainable and nutritional alternative.....	86
▪ Incorporation of <i>Acheta domesticus</i> powder in sauces: A sustainable and nutritional alternative	87
▪ Exploring the effect of feeding and production batches on the microbiological safety of <i>Tenebrio molitor</i> larvae.....	88
▪ Formation of <i>Acinetobacter</i> biofilms on stainless steel: risks for food safety and industrial contamination	89
▪ Impact of <i>Escherichia coli</i> on the Growth of <i>Listeria monocytogenes</i> : A Preliminary Assessment	90
▪ Agile methodologies used to design healthy and sustainable food in SMEs in Iberoamerica.....	91
▪ Quality and microbial profile of chouriço: influence of salt reduction	92
▪ Making <i>garum</i> in Troia in the 21 st century.....	93
▪ Impact of Refrigeration on the viability of <i>Campylobacter</i> spp. in water	94
▪ Cold Survival: Does strain origin affect the growth of <i>Listeria monocytogenes</i> in milk at storage temperatures?	95
▪ Consumers' acceptance and conceptualisation of innovative food products with edible algae	96
▪ Encapsulated aromatic oleoresins and KCl as NaCl replacements in meat products	97
▪ Enhancing catfish (<i>Clarias gariepinus</i>) fillet quality through optimized salting and smoking techniques	98
▪ <i>Listeria monocytogenes</i> : Dead or Just Pretending? Unveiling the VBNC State	99
▪ Impact of cognitive style on the evaluation of consumer perception of different orange juices: a case study with projective mapping approach	100
▪ Effect of storage time and temperature on the acceptability of canned mackerel with vegetables	101
▪ Sensory evaluation and consumer liking of bread enriched with <i>Tenebrio Molitor</i> flour	102
▪ The influence of typefaces on the emotional response and sensory evaluation of food products.....	103
▪ Effect of mixing alternative proteins on the techno-functional properties of the resulting mixtures.....	104
▪ Development of functional insect-based food products: insights from a market study	105
▪ 'Maçã de Alcobaça' PGI: determinants of choice by Portuguese consumers	106

- Innovation in the technological production of aged honey spirit with acacia wood 107
- Co-Processed olive oils with culinary herbs: a new food preparation 108
- The impact of ingredient concentration on the stability of basil essential oil nanoemulsions 109
- Survival and proliferation of foodborne pathogens in mung bean sprouts irrigated with contaminated water 110
- Assessment of quality factors and shelf life of industrially processed fresh-cut pineapple 111
- Exquisite cuisine: development of novel pudding and mousse recipes based on red macroalgae 112
- Characterization of the nutritional composition of four macroalgae species from the Western Portuguese coast to food diet alternatives 113
- Design of innovative electrochemical genosensors for honey fraud and quality detection by botanical origin authentication 114
- Assessing the nutritional potential of *Undaria pinnatifida* and *Himanthalia elongata* for human consumption 115
- Antioxidant potential of seaweed extracts in fish oil preservation 116
- Influence of drying method and packaging material on the shelf life of orange-fleshed sweet potato flour 117
- Choux pastry - the role of extrusion for improved pre-gelatinised wheat flour 118
- Conception, development and construction of an Industrial-scale pilot-plant for desinfestation of chestnuts 119
- Genopheno4trait project - genomic and phenotypic traits contributing to persistence of *listeria monocytogenes* in food processing environment 120

NUTRITION, HEALTH AND WELLBEING..... 121

- Fortification of White Wine Vinegar with Anthocyanin-Rich Edible Flowers: Effects on Polyphenol Content, Aromatic Profile, and Sensorial Properties 122
- Nutritional and Contaminant Analysis of Foods Ingested by Vegetarian Consumers: Total Diet Study Approach 123
- Fostering Smart Nutrition: mitigating the effects of child food insecurity 124
- From carob pulp to cereal bowls: A functional flour for wellness and sustainability 125
- Promising Buccal Films Containing *Actinidia Arguta* Fruit Extract for Oral Mucositis Treatment: Characterization and *In Vivo* and *Ex Vivo* Permeation Profile 126
- *In vitro* evaluation of the antibacterial activity of putative probiotic strains against enterotoxigenic *Escherichia coli* 127
- *In vitro* digestion and bioaccessibility studies of Vitamin D3 in Chitosan/Gum Arabic particles' stabilized 3D printable Pickering Emulsions 128

- Polyphenols as enhancers of emulsifying and antioxidant properties of egg and yeast protein emulsions: understanding the molecular interactions behind their technological and functional potential..... 129
- Insects as a novel and functional food source to prevent hypertension: the case of house cricket..... 130
- Challenges in food allergen quantification: DNA versus proteins..... 131
- Can food processing and digestibility impact the allergenicity of shellfish and insect foods? 132
- Nanoplate digital PCR for food quality and safety testing: application to botanical authentication of bacopa food supplements 133
- Towards trending functional foods: combining spirulina with alternative protein sources 134
- Biosensors as pioneering solutions for monitoring food allergens and diagnosing food allergy 135
- Selection of aptamers to target enterotoxigenic *Escherichia coli* infections 136
- Insects as a sustainable alternative to animal protein: assessing digestibility, allergenicity and inflammatory potential..... 137
- Evaluating children's preferences for sustainable menus in school canteen' 138
- Hemp seeds and their role in promoting health and sustainability 139
- Impact of cellulose nanocrystals as a new food ingredient on microbiota health . 140
- Sweet-tasting oligosaccharides as a strategy for functional sugar reduction in foods 141
- Determining the LC₅₀ of hydro-ethanolic extract of *Eriobotrya japonica* leaves from Azores region on zebrafish embryos for toxicological insights..... 142
- Giving a second life to winery by-products: development of crackers formulation as a health promoter 143
- Strategies to increase prebiotic activity and neurotrophic potential through dietary fibre modulation 144
- Understanding the motives behind the consumption of cow's milk and its plant-based substitutes in two contrasting European markets 145
- Impact of previous exposure to fraud on Portuguese consumer perceptions and attitudes towards food fraud 146
- Exploring Phenolic Content and Antioxidant Properties of Enzyme-assisted Extracts from Red Seaweed *Porphyra dioica*..... 147
- Germinated quinoa (*Chenopodium quinoa*): Effects on mineral composition, antioxidant capacity, and technofunctional properties 148
- Development of 3D-printed structured food models using 149
- Chemical characterization of *Agaricus bisporus* by-products for development of functional ingredients 150
- Coffee brew can aid on fight against cholesterol? 151

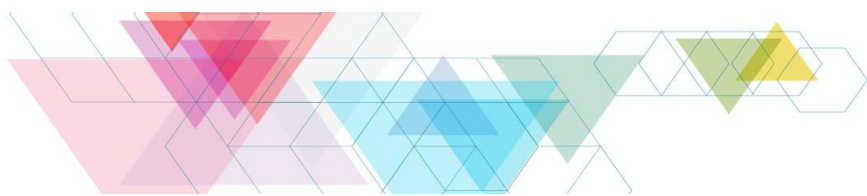
- Culinary Plant-Based Decoctions: A promising approach for combatting neurodegenerative disorders..... 152
- Fatty acid profile and antioxidant activity of andiroba (*Carapa guianensis* Aubl.) seed oil 153
- Swine Industry: Validated PCR-based method for detecting enterotoxigenic *Escherichia coli* 154
- Replacing inorganic phosphate in clean-label cooked hams 155
- Fate of (poly)phenols from radish microgreens grown under different lights: Digestion and gut microbiota catabolism..... 156

SUSTAINABILITY AND CIRCULAR ECONOMY 157

- Roll-Pulp: Closing the loop on apple juice coproducts 158
- Food by-products recycling towards production of eco-friendly pH-responsive membranes using natural-inspired dyes..... 159
- Development of carbon dot-embedded polymeric films for intelligent packaging applications 160
- Harnessing black soldier fly larvae for sustainable food waste valorisation and pathogen bioremediation..... 161
- Olive Pits: From agriculture waste to a value product..... 162
- Functional Muffins with Lime Extract: A Step Toward Sustainable Food Innovation 163
- Aligning present and future agrifood systems: Integrating sustainability and health for better choices..... 164
- From grape pomace to valuable products: Sequential fractionation using ohmic heating and subcritical hydrolysis..... 165
- Sustainable value chains in mountain rural areas: 166
- Strategies for a model region 166
- Sustainable production of lignin nanoparticles from grape stalks via sonication .. 167
- Comparative assessment of solid and dissolved lignin from grape stalks for antibacterial applications..... 168
- Linking Landscape, Environment, Agriculture and Food towards a more sustainable agri-food value chain..... 169
- Valorizing saffron by-products as a source of an anthocyanin-rich natural colourant 170
- Circular Animal Protein Hydrolysates: a comparative approach of functional properties..... 171
- Evaluating the Influence of Functional Unit Selection in Life Cycle Assessment: A Bread Case Study..... 172
- Chestnut Burrs: A cellulose source for stabilising pickering emulsions 173
- Extraction and stabilisation of bioactive compounds from chestnut shell residues 174

- Ohmic heating extraction of pectin from cacao shells: A sustainable and efficient approach for agroindustrial waste valorization..... 175
- Exploring agro-industrial by-products for bioactive potential in food packaging: A study on *Punica granatum L.* and *Castanea sativa* 176
- Assessment of olive oil industry by-products as a source of phytoprotective agents for agriculture 177
- Olive oil byproducts extracts: Bioactive potential in advanced cell models of intestinal inflammation and colorectal cancer 178
- *In Vitro* and *In planta* screening of bacterial control agents against Pear Scab with Imaging and molecular detection methods 179
- Biocontrol potential of novel rhizospheric bacterial strains against *botrytis cinerea*: Functional validation and genomic insights 180
- Valorisation of wine by-products: Extraction and characterization of antioxidants for food applications 181
- Cork extracts as bioactive agents: Antioxidant potential and formulation strategies 182
- INNOECOFOOD – Eco-Innovative technologies for improved nutrition, sustainable production and marketing of agroecological food products in Africa 183
- NEWPACK - A New Generation of Packaging Materials 184
- Organizational Life Cycle Assessment of innovation centre for agri food industry: Evaluating the carbon footprint of a colab 185
- An industrial waste stream to a final product: The valorisation and incorporation of a tomato ketchup by-product..... 186
- Up-cycling broccoli leaves: lactic acid fermentation as a sustainable approach to reduce food loss..... 187
- Enzymatic modelling of juice production pomace for novel ingredient development in non-dairy infant nutrition..... 188
- Winery by-products as a source of bioactive phenolics: antioxidant and enzymatic inhibitory properties..... 189
- Unlocking value of marine co-products through fish protein hydrolysates..... 190
- Study of the phenolic composition of wines from grapevines irrigated with treated wastewater..... 191
- Impact of non-thermal technologies on whey stabilization at pilot scale 192
- From winery waste to oral health: Grape stem polyphenols as a sustainable treatment for periodontal diseases 193
- Stabilization of watermelon albedo and evaluation of its potential as a dehydrated snack 194
- Active films incorporating α -tocopherol in polymeric matrix by extrusion and ultrasonic spray coating 195
- Characterisation of apple and ginger pomace for potential syrup production: Nutritional composition, antioxidant capacity, and reducing sugar profile 196

▪ Consumer perception of a new sustainable food product made from discarded table grapes.....	197
▪ Valorisation of grape stems: Polyphenolic profile and inhibition of skin-aging enzymes for cosmetic and pharmaceutical applications	198
▪ Impact of previous exposure to fraud on Portuguese consumer perceptions and attitudes towards food fraud	199
▪ Control of <i>Stemphylium vesicarium</i> in “Rocha” Pear Using Encapsulated Essential Oils	200
▪ From Waste to Value: Antioxidant and Antimicrobial Properties of Fresh and Dried Cardoon Leaf Extracts	201
▪ Turning agri-food by-products into functional ingredients: sustainable innovations for waste reduction.....	202
▪ Incorporating chestnut shell fibers into biodegradable films for enhanced performance.....	203
▪ Brewing Spent Yeast as a Sustainable Solution for Tannin Adsorption	204
▪ Upcycling spent coffee grounds: Green synthesis of nZVI for soil and water remediation	205
▪ From waste to value: Environmental impact assessment of chestnut-based candle holders.....	206
▪ Phytochemical and biotechnological valorisation of winery by-products for prostate cancer cell modulation	207
▪ Pumpkin power: Turning butternut by-products into functional food gold.....	208
PROJECTS	209
▪ MICROORC: Orchestrating food system microbiomes to minimize food waste ...	210
▪ PAS-GRAS - De-risking metabolic, environmental and behavioral determinants of obesity in children, adolescents and young adults.....	212
▪ Food Chase - Food supply-chain ecosystems for sustainability	213



SPONSORSHIPS

Main Partner



Hot



Spicy



Mild



StartUp



Apoios



Apoio Institucional



AGRICULTURA E PISCAS



Apoio Científico



Apoio Media



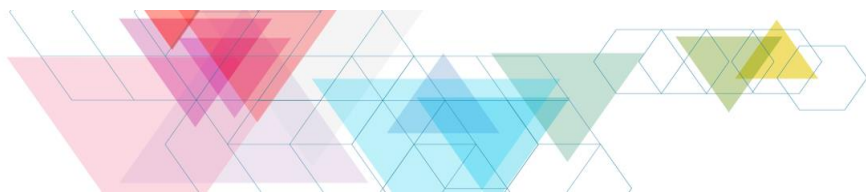
Authors: Ana Patrícia Sousa, Alexandra Pedro, Andreia Silva, Joana Calmeiro, José Evaristo, Miguel Teixeira, Carina Almeida, Deolinda Silva.

Edition: Associação Colab4Food - Laboratório Colaborativo Para Inovação da Indústria AgroAlimentar

E-mail: info@dare2change.com

April 2025 © Dare2Change®

This publication brings together the abstracts of the poster communications presented at the Dare2Change® 2025. All abstracts were evaluated by the Scientific Committee of the Conference.



ORGANIZING COMMITTEE

Amândio Santos, *Grupo Primor, S.A. - Presidente do Conselho de Administração – PortugalFoods & Colab4Food*

Carina Almeida, *Instituto Nacional de Investigação Agrária e Veterinária, I.P.*

Deolinda Silva, *PortugalFoods*

Miguel Teixeira, *Colab4Food*

Nuno Canada, *Instituto Nacional de Investigação Agrária e Veterinária, I.P.*

OPERATIONAL TEAM

Alexandra Pedro, *Colab4Food*

Ana Sousa, *Colab4Food*

Andreia Silva, *Colab4Food*

Francisco Gândara, *Colab4Food*

José Evaristo, *Colab4Food*

Margarida Santos, *PortugalFoods*

Teresa Carvalho, *PortugalFoods*

SCIENTIFIC COMMITTEE

Ana Barros, *Universidade de Trás-os-Montes e Alto Douro*

Anabela Raymundo, *Universidade de Lisboa*

Carina Almeida, *Instituto Nacional de Investigação Agrária e Veterinária, I.P.*

Cristina Matos, *Instituto Politécnico do Porto*

Inês Coelho, *Instituto Nacional de Saúde Doutor Ricardo Jorge*

Isabel Ferreira, *Universidade do Porto*

Jorge Noro, *Universidade de Coimbra*

José Teixeira, *Universidade do Minho*

Lorenzo Pastrana, *International Iberian Nanotechnology Laboratory*

Manuel Coimbra, *Universidade de Aveiro*

Manuela Vaz Velho, *CISAS - Instituto Politécnico de Viana do Castelo*

Paula Teixeira, *Universidade Católica Portuguesa*

Victor Freitas, *Universidade do Porto*

ADVISORY COMMITTEE

Ana Gomes, *Universidade Católica Portuguesa*

Anabela Raymundo, *Universidade de Lisboa*

Paulo Marques, *Sumol + Compal Marcas, S.A*

Vítor Hugo Gonçalves, *WaterBunkers SGPS S.A*

PROGRAM

08h30 | Receção dos participantes

09h00 | Sessão de boas-vindas

CONTEXTO GLOBAL PARA O NEGÓCIO AGROALIMENTAR

09h15 | Os desafios da política ao comércio internacional

Filipe Vasconcelos Romão, Professor da Universidade Autónoma de Lisboa

09h30 | A procura de valor acrescentado nos mercados internacionais

Gonçalo Andrade, Presidente da Portugal Fresh

09h45 | Internacionalizar a inovação no agroalimentar

Ricardo Arroja, Presidente do Conselho de Administração da AICEP

10h00 | Debate

Moderação: Filipe Vasconcelos Romão, Professor da Universidade Autónoma de Lisboa

Intervenção do Secretário de Estado da Agricultura, João Moura

DARE2SNACK Momento com as start-ups Micro Harvest e Brainr

O FUTURO: ALIMENTAÇÃO E SUSTENTABILIDADE

11h00 | Conciliar tradição, inovação e saúde no desenvolvimento da estratégia alimentar nacional

Pedro Graça, Professor Associado da Universidade do Porto e Diretor da FCNAUP

11h15 | Alterações legislativas nas embalagens

Mário Raposo, CEO da NOWA

11h30 | **Moderação:** Alexandra Bento, Coordenadora do Departamento de Alimentação e Nutrição do INSA

DARE2CONNECT

12h00 | ANIL - Matriz Láctea: Importância e Aplicação Atual no Contexto da Nutrição e Saúde

Maria Cândida Marramaque, Directora-Geral da ANIL

12h15 | ANICP - Longevidade e Qualidade de Vida

Carina Castro Silva, Médica de Saúde Pública e Carlos Cardoso, Investigador no IPMA

12h30 | APIC - O que falta comunicar sobre setor da carne

Graça Mariano, Diretora Executiva da APIC

DARE2LUNCH

DISRUPÇÃO ALIMENTAR

14h30 | Tasting Tomorrow: Innovation, Creativity, and Food Design

Ricardo Bonacho, Co-fundador do Food Design Lab Lisboa

14h45 | A industrialização da carne cultivada: sonho ou realidade? O exemplo da Meatly

Helder Cruz, Co-fundador, Chief Scientific Officer e Membro do Board da Meatly

15h00 | Innovating for market success

John Melo, CEO da Digestiva

15h15 | Debate

Moderação: Nuno Gama, Diretor Executivo e Membro do Conselho de Administração do PORTIC

DARE2SNACK Momento com as start-ups Corial e Sensesbit

PERFORMANCE & COMPETITIVIDADE

16h15 | Cerealis, Transformar para Crescer

Pedro Moreira da Silva, CEO da Cerealis

16h35 | Performance individual e das equipas

José Soares, Professor Catedrático da Universidade do Porto

SESSÃO DE ENCERRAMENTO

17h00 | Entrega de prémios para os melhores pósteres científicos

FAREWELL BEER EXPERIENCE BY SUPER BOCK GROUP



Abstracts of Poster Presentations

Digitalization and AI in the Agri-Food Sector

Building a community of practice to CATALYSE food safety breakthroughs

Diana R. Fonseca, João Cortez, Paula Teixeira

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina –
Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005
Porto, Portugal
dpfonseca@ucp.pt; jcortez@ucp.pt; pcteixeira@ucp.pt

Contaminated food causes 600M cases of foodborne diseases and 420k deaths annually, resulting in 33M years of healthy lives lost¹. This is probably an underestimation, but these numbers could be significantly reduced if advances and innovations in food safety were effectively promoted and widely implemented. However, barriers such as limited collaboration, complex regulations, and high costs hinder the adoption of improved practices.

To address these challenges, the European project CATALYSE seeks to catalyse scientific innovations into practical food safety actions by creating a Community of Practice (CoP). A CoP unites people and organizations with a shared interest in a domain who collaborate to exchange knowledge, solve problems, and advance innovation². The CATALYSE CoP aims to foster collaboration and knowledge sharing between food safety actors to accelerate the adoption and scaling-up of innovative practices and technologies. This contributes to a more resilient, sustainable, and equitable community, meeting the needs of all stakeholders, from “farm2fork”, with the common goal of ensuring food safety. Educational tools will be developed to fulfill stakeholders needs and the active dissemination of new knowledge and innovative solutions generated by the scientific and industry communities will be promoted. This collaborative online platform will facilitate dialogue and collaboration across the whole food system, leading to win-win solutions for all stakeholders involved. CATALYSE will not generate science by itself or promote specific proprietary solutions: it will be a common online place where all the actors of the food chain can meet and define together the needs and possibilities that innovation can answer and/or offer. We invite you to join our CoP to connect with peers, access cutting-edge resources, and contribute to the development of safe and sustainable food systems. By becoming a member of CATALYSE CoP, you will have access to consolidated updates on food safety, all in one place.

Acknowledgments: Grant Agreement:101136754 – Ref. Ares (2023)7542280 - 07/11/2023

References:

- [1] WHO estimates of the global burden of foodborne diseases: Foodborne Disease Burden Epidemiology Reference Group 2007–2015 (World Health Organization)
- [2] Wenger, E. (1998), *Communities of Practice: Learning, Meaning and Identity*, Cambridge University Press, Cambridge.

High-quality high-impact augmented virtuality system for the evaluation of the impact of context on consumer perception and hedonics

João Pedro Marques^{1,2,3}, José Carlos Ribeiro², Rui Costa Lima¹, Célia Rocha^{1,2}, Luís Miguel Cunha^{2*}

¹ Sense Test, Vila Nova de Gaia, Portugal

² GreenUPorto/Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Porto, Portugal

³ Faculty of Nutrition and Food Science of the University of Porto, Porto, Portugal

* Imcunha@fc.up.pt

Context is a critical factor in sensory and consumer science, influencing perceptions, emotions, and hedonic evaluations of food products [1]. Traditional sensory analysis, conducted in neutral booths, often lacks ecological and external validity due to the absence of environmental cues, leading to differences between lab-based and real-world consumer responses [2,3,4]. Advancements in digital technologies have led to the development of novel evaluation methodologies, including immersive approaches [5,6]. Among these, Augmented Virtuality (AV), a subset of Mixed Reality, merges virtual simulations with real-world elements, enhancing realism and user engagement [6].

Hence, this work presents a high-quality AV system developed in Unity (Unity Technologies, version 2022.3.42), designed to perform large-scale sensory and consumer tests with a high-degree of immersion and realism, while enabling user-friendly data collection. The system utilizes Varjo XR-4 (Varjo Technologies) head-mounted displays (HMDs) to present 8K (8192 x 4096) 360° videos of real environments (e.g., pub, beach bar) projected onto a chromakey area, ensuring a high-degree of realism. Simultaneously, real-world items (e.g., food, utensils, mobile device, participant's hands) are visible through the HMD, providing a seamless integration of virtual and physical elements. Additionally, precise spatial tracking of both the participants and the evaluation table setup is ensured by base stations (HTC Vive, SteamVR Base Station 2.0) and markers, respectively. Real-time data collection is supported through API-synchronized questionnaires on mobile devices, with audio prompts also delivered through the HMD, ensuring a seamless and immersive experience for participants.

Looking forward, this AV solution has the potential to revolutionize sensory and consumer research by improving ecological and external validity, reducing operational costs associated with context research, and enabling immersive, high-engagement studies across diverse food consumption environments. Practical applications include market testing and personalised consumer experiences, opening the way for a new era in sensory and consumer science, specifically on context-driven product evaluations.

Acknowledgments: João Pedro Marques acknowledges Non-Academic Doctoral grant no. 2023.03845.BDANA, funded by the Foundation for Science and Technology (FCT) and supported by Sense Test, Lda. Authors acknowledge support from the Mobilizing Agenda "VIIAFOOD" from the Portuguese PRR (Aviso Nº 02/C05-i01/2022) with No. C644929456-00000040, under the R&D project "HIT-C - High Integration Technology for Consumer Insights". Authors João Pedro Marques, José Carlos Ribeiro, Célia Rocha and Luís Miguel Cunha are thankful to national funds via FCT through UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável.

References:

- [1] H. L. Meiselman, The future in sensory/consumer research: Evolving to a better science, *Food Quality and Preference*, 27 (2013) 208–214.
- [2] E. P. Koster, The psychology of food choice: Some often encountered fallacies, *Food Quality and Preference*, 14 (2003) 359–373.
- [3] A. Stelick & R. Dando, Thinking outside the booth—The eating environment, context and ecological validity in sensory and consumer research, *Current Opinion in Food Science*, 21 (2018) 26–31.
- [4] P. García-Segovia, R. J. Harrington, H.-S. Seo, Influences of table setting and eating location on food acceptance and intake, *Food Quality and Preference*, 39 (2015) 1–7.
- [5] A. Galiñanes Plaza, J. Delarue, L. Saulais, The pursuit of ecological validity through contextual methodologies. *Food Quality and Preference*. 73 (2019), 226–247.
- [6] M. Liberatore & W. Wagner, Virtual, mixed, and augmented reality: a systematic review for immersive systems research. *Virtual Reality*, 25 (2021) 1–27.

Gamified online training to improve motivation and knowledge retention in food industry training

Jairo Torres^{1,*}, Letricia Barbosa-Pereira¹, Joao Lima², Adres Escobar³, María Cortes³, Hernán Pávez³, Nicolas Espinoza⁴, Angelica Botero⁵

¹FoodChemPack Research Group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain,

²H&TRC—Health & Technology Research Center, Coimbra Health School, Polytechnic University of Coimbra, 3045-043 Coimbra, Portugal; GreenUPorto - Sustainable Agrifood Production Research Centre, Porto, Portugal; SUScita - Research Group on Sustainability, Cities and Urban Intelligence, Coimbra, Portugal,

³HILLUS Comunicaciones SPA. Santiago, Chile,

⁴Fundación desarrollo educativo- observatorio de juego. Santiago, Chile,

⁵Alimentos Nebraska S.A. La Unión, Colombia.

* *jairoalonso.torres@rai.usc.es*

Introduction: Training operational staff in the food industry is challenging due to work schedules and low motivation. Traditional and face-to-face training methods have limited coverage and reduced knowledge retention. This research aims to identify whether online training methodologies, with gamified audiovisual resources, can be an effective alternative to complement staff training.

Method: A case study was conducted in a food industry company in Colombia with 82 employees. Four courses were designed and developed according to the company's needs, covering the following topics: cleaning and disinfection, efficient water use, allergen management, and cardiovascular risk prevention. Multimedia resources, such as micro-capsules, podcasts, interactive activities, notes, and infographics, were used in a gamified environment on the Moodle platform developed for mobile devices. Tests were conducted to measure knowledge retention, and questionnaires and focus groups were used to assess user satisfaction.

Results: The courses were completed by 43 administrative employees and 39 production staff, with a distribution of 36 women and 46 men. Of these, 48% had technical or university-level education, while 52% had basic education. The average time to complete each course was 4.5 days. The knowledge retention rate was 80%. The users' satisfaction level was high, particularly with the content and multimedia resources used. Suggested improvements included making chapters shorter, improving the user experience by optimizing the platform, familiarizing users with the platform, and increasing interactivity.

Conclusion: The use of online courses with gamified audiovisual resources can be an effective alternative to enhance motivation and coverage of online training for operational staff in the food industry.

References:

- [1] B.-E. Grass, M. Coto, C.-A. Collazos-Ordoñez, P. Paderewski, "Learning about Programming and Epistemic Emotions: A Gendered Analysis," *Revista Facultad de Ingeniería*, vol. 29 (54), e12034, 2020.
- [2] SILVA, Guilmer Brito. MOOCs gamificados: propuesta de diseño pedagógico para cursos online. 2019.151 y sig. Tesis (Doctorado en Educación) – Núcleo de Educación, Programa de Postgrado en Educación, Universidad Federal de Alagoas, Maceió, 2019
- [3] Terán Bustamante, Antonia, & Mendieta Jiménez, Bertha. (2019). Modelo de transferencia de conocimiento a través de la gamificación: un gcMooc. *Actualidades Investigativas en Educación*, 19(2), 105-131.
- [4] Martín, Martinha, y Costa, Carlos J. (2017). CANOE y Flow: Determinantes en la adopción de un curso de programación online gamificado. *RISTI - Revista Ibérica de Sistemas y Tecnologías de Información*, (25), 34-53



Food Development and Production

Developing plant-based protein formulations as alternatives to egg white protein in vegan ham-like products

Ana Rita Pereira, Ana Fernandes*, Nuno Mateus, Victor de Freitas

LAQV-REQUIMTE, Faculty of Sciences, University of Porto, Porto, Portugal

* ana.fernandes@fc.up.pt

In a world where the lack of resources expected to meet the demands of a growing population is increasingly evident, new alternatives have been explored by the Food Industry. Egg white protein (EWP) is widely used in conventional cured meat analogs for its excellent texturizing and binding properties. However, plant-based meat analogs, crafted mainly from legumes, soybeans, wheat, and lentils [1], have been explored due to health, sustainability, and animal welfare concerns. Additionally, the by-products of the vegetable and fruit industry can be used as technological ingredients due to their rich composition in bioactive compounds such as dietary fibers and polyphenols [2]. Yeast protein extract (YPE) and β -glucans, known for their versatile functional properties, have gained attention as fortifying ingredients. YPE is rich in easily digestible proteins, amino acids, and natural umami enhancers [3], and β -glucans are recognized for their role as hydrocolloids, offering thickening, stabilizing, and texturizing effects in food formulations [4]. This study aimed to replace EWP with mung bean protein and evaluate the potential of YPE and β -glucans (in solution and powder) as texturizer ingredients. Additionally, the effect of water-oil/protein ratios was assessed. The ham-like formulation was based on a protocol supplied by the Primor Group and evaluated considering the Texture Profile Analysis (TPA) and CIELab color coordinates. Initial results revealed that mung bean protein significantly reduces the texture parameters, including decreases of approximately 70% in hardness and elasticity, and 90% in chewiness compared to the EWP control, presenting a soft texture. YPE incorporation improved the texture properties, particularly when the water-to-oil/protein ratio was reduced, resulting in higher hardness values, even though the improvements were insufficient to fully match the EWP control. Further enhancements were achieved by incorporating β -glucans, resulting in an increased hardness of 60% compared to mung bean protein and 20% compared to YPE, with slight increases in elasticity and chewiness. Powdered β -glucans demonstrated promising results, achieving hardness values comparable to the EWP control. Cohesiveness and elasticity remained closer to EWP control. Although the chromatic analysis revealed some notable differences from the control, the formulations still showed appealing colors.

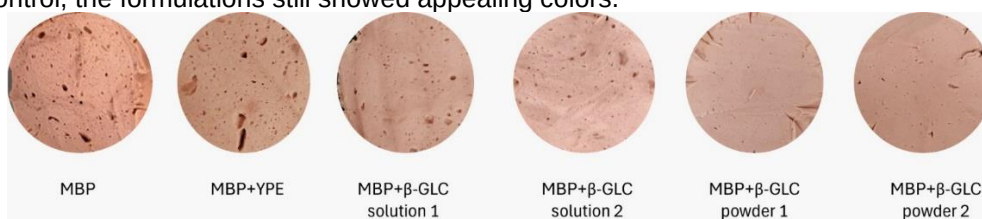


Figure 1. Formulations based on mung bean protein (MBP) incorporating Yeast Protein Extract (YPE) and β -Glucan (β -GLC) in solution and powder at varying concentrations.

Funding: This work received financial support from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

Acknowledgments: The authors acknowledge the project VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar: PRR - C644929456-00000040 – VIIAFOOD, to Primor Group for their collaboration and support, and to Proenol for supplying Divino (YPE) and β -glucans. AF acknowledges FCT for the research contract (CEECIND/00029/2018/CP1545/CT0010).

References:

- [1] Kyriakopoulou K., Keppler J.K., van der Goot A, *Foods*. 2021;10:600.
- [2] Fernandes, A., N. Mateus, and V. de Freitas, *Foods*, 2023. 12(5): p. 1052.
- [3] Wang H, Wang W, et al. *Food Chemistry* (2023);15:429:136807.
- [4] Jurkaninová L, Dvořáček V, Gregusová V, Havrlentová M, *Foods*, 2024. 13(3):500.

3D Printing vegan snack: A sustainable innovation in food production

**Lisiane Carvalho^{1*}, Leonor Pereira¹, Adriana Ferreira², Anabela Raymundo¹,
Teresa Matos¹, Isabel Sousa¹**

¹ LEAF - Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

² R&D Departament, Primor Charcutaria-Prima, S.A., Gavião, 4760-003 Vila Nova de Famalicão, Portugal.

* *isabelsousa@isa.ulisboa.pt*

Meat production significantly impacts the environment due to greenhouse gas emissions and the extensive use of water and land resources. Allied to this, the growing population and demand for animal protein hinders the reduction of these impacts^{1,2}. Consequently, many people are reducing their consumption of animal-based products, while food companies develop plant-based meat alternatives³. 3D printing technology in food production creates products with personalized nutrition and appealing textures, offering consumers an attractive alternative^{4,5}. This study aimed to develop a vegan snack using 3D printing technology and analyses its texture and nutritional composition.

Instead of meat, isolated pea and potato proteins were used, with various ingredients in different formulations and tuned through rheology properties to ensure printability. A thermomixer was used to mix pre-gelled xanthan and LBG systems with other ingredients. The dough was printed using a 3D printer (Genesis TM, 3D Biotechnology Solutions, São Paulo, Brazil) with 100% fill, a 0.8 mm nozzle diameter, 180% print speed (72 mm/min), and 180% flow rate. The snack was baked at 125°C for 15 minutes and then cooled to room temperature.

The texture analysis showed the snack had higher firmness in the cutting test (2.5 N) than in the penetration test (0.9 N), likely due to the cutting blade's sensitivity. Nutrient composition showed 44% moisture, 6% ash, 8% lipids, 32% protein, and 11% carbohydrates (calculated by difference). According to Regulation (EC) n° 1924/2006, the claims "high in protein" and "rich in minerals" phosphorus (238.7 mg/100g), iron (9.4 mg/100g) and copper (0.3 mg/100g), as well as a "source of" zinc (2.5 mg/100g) and manganese (0.3 mg/100g) can be applied. Total phenolic compounds were 0.32 mgGAE/g and antioxidant activity was 0.1 mgTE/g.

The study's results suggest that 3D-printed snacks could promote sustainable, nutritionally tailored foods, reducing reliance on animal proteins and meeting current consumption needs.

Acknowledgments: This work was supported by Agendas Mobilizadoras para a Inovação Empresarial - PRR I Aviso No 02/C05-i01/2022 project VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040). WP3 – Vegetarian/Vegan Meat Analogues Products. Linking Landscape, Environment, Agriculture and Food – LEAF (UIDB/ 04129/ 2020). Primor Charcutaria-Prima, S.A.

References:

- [1] R. R. Balandrán-Quintana, A. M. Mendoza-Wilson, G. Ramos-Clamont Montfort, J. Á. Huerta-Ocampo, Proteins: Sustainable Source, Processing and Applications, (2019) 97–130.
- [2] L. H. Fasolin, R. N. Pereira, A. C. Pinheiro, J. T. Martins, C. C. P. Andrade, O. L. Ramos, A. A. Vicente, Food Research International, 125 (2019) 108586.
- [3] C. Graça, A. Raymundo, I. de Sousa, LWT, 74 (2016) 263–270.
- [4] E. G. Bulut, K. Candoğan, LWT, 154 (2022) 112768.
- [5] S. Oliveira, I. Sousa, A. Raymundo, Algal Research, 68 (2022) 102879.

Impact of thermal treatment on *Talaromyces trachyspermus* spores inactivation

Sousa, D.^{1,2,*}, Noronha, L.³, Venâncio, A.^{1,4}, Silva S. C.^{1,2,4}

¹ CEB - Centre of Biological Engineering, UMinho

² INIAV - National Institute of Agrarian and Veterinary Research, Vairão, Portugal

³ Colab4Food - Collaborative Laboratory for Innovation in the Agri-Food Industry, Vairão, Portugal

⁴ LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

*dianasousa14@hotmail.com

Heat-resistant moulds (HRMs) are microorganisms recognized for their ability to endure high temperatures encountered during heat treatment [1,2]. HRMs exhibit both an asexual phase, characterized by the production of non-heat-resistant spores called conidia, and a sexual phase, which generates heat-resistant ascospores [3]. These ascospores are formed within asci, many of which are enclosed in a large fruiting body referred to as an ascocarp [4]. In their natural state, ascospores remain dormant and require activation by external triggering stimulus, such as heat or high pressure, to germinate [5]. The spoilage resulting from HRMs presents a major challenge for the fruit juice industry, resulting in considerable economic losses and a key issue in this sector is the presence of *Talaromyces trachyspermus*.

Thus, the aim of this work was to evaluate the effect of thermal efficacy on the inactivation of conidia and ascospores produced by *T. trachyspermus*.

The spores of *T. trachyspermus* were produced on malt extract agar under different incubation periods of 1 week, 1 month, 2 months, and 6 months at 25 °C. Thermal treatments ranging from 45 °C to 70 °C for up to 15 minutes were conducted to determine the temperature required to inhibit conidia and activate heat-resistant ascospores.

Results showed that conidia were completely inactivated at 55 °C after 10 minutes of exposure, regardless of the incubation period. In contrast, ascospores exhibited greater thermal resistance, maintaining their viability, with evidence of thermal activation at 70 °C. Prolonged incubation periods (2–6 months) increased the thermal resistance of both conidia and ascospores. These findings suggest that while conidia are heat-sensitive and can be effectively inactivated at 55 °C, ascospores are thermoresistant and can be activated under the pasteurization conditions used industrially. The study underscores the need for rigorous thermal treatments to control *T. trachyspermus* spores in industrial settings, particularly during pasteurization processes.

Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, with DOI 10.54499/UIDB/04469/2020, by LABBELS - Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020, by the National Institute for Agrarian and Veterinary Research (INIAV), by Colab4Food, and by SUMOL+COMPAL Marcas, S.A. Diana Sousa also acknowledges FCT for the fellowship 2023.04050.BDANA.

References:

- [1] Doğan E, Demirel Zorba NN. Heat-Resistant Moulds in Fruits and Fruit-Containing Products. *Journal of Fungus* 2024.
- [2] Santos JLP dos, Samapundo S, Biyikli A, Van Impe J, Akkermans S, Höfte M, et al. Occurrence, distribution and contamination levels of heat-resistant moulds throughout the processing of pasteurized high-acid fruit products. *Int J Food Microbiol* 2018;281:72–81..
- [3] Maneeboon T, Sangchote S, Hongprayoon R, Chuaysrinule C, Mahakarnchanakul W. Occurrence of Heat-Resistant Mold Ascospores in Pineapple and Sugarcane Field Soils in Thailand. *Int J Microbiol* 2023;2023:1–13.
- [4] Stiers DL. The fine structure of ascospore formation in *Ceratocystis fimbriata*. *Canadian Journal of Botany* 1976;54:1714–23.
- [5] Dijksterhuis J, Teunissen PGM. Dormant ascospores of *Talaromyces macrosporus* are activated to germinate after treatment with ultra high pressure. *J Appl Microbiol* 2004;96:162–9.

Killer yeasts as sustainable solutions for foodborne pathogens control

Patrícia Serra^{1,2*}, Daniela Araújo², Fernanda Gomes^{2,3}, Cristina Almeida Aguiar^{4,5}, Sónia Silva^{2,3}

¹ INIAV - National Institute for Agrarian and Veterinarian Research, Vairão, Portugal

² CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal

³ LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

⁴ DB- Departamento de Biologia, Universidade do Minho

⁵ CBMA- Centro de Biologia Molecular e Ambiental, Universidade do Minho

* pmserra02@gmail.com

Killer yeasts produce toxins that can effectively suppress other microorganisms, providing a natural competitive advantage across diverse environments [1]. This phenotype establishes yeasts as highly promising biocontrol agents for combating spoilage organisms and foodborne pathogens [2]. In this study, yeast strains isolated from raw milk in the northern dairy region of Portugal were evaluated for their killer activity under different pH and temperature conditions. Initially, using a cross-reaction protocol with yeast strains from different species, it was observed that the effectiveness of killer activity was influenced by environmental conditions, with higher efficacy at acidic pH (3 and 4) and lower temperatures (20–25 °C). This screening revealed that *Clavispora lusitaniae*, *Issatchenkia* spp., *Trichophyton erinacei*, *Fungal endophyte*, *Candida tropicalis*, and *Cyberlindnera jadinii* exhibited the strongest killer activity. To investigate the potential of these killer yeasts as sustainable solutions for controlling foodborne pathogens, we tested their effectiveness against common bacterial contaminants. The findings showed that *Cyberlindnera jadinii* displayed considerable potential as a killer against *Escherichia*, *Salmonella*, and *Proteus* species. These findings highlight the efficacy of killer yeasts as biocontrol agents, capable of addressing microbial contamination in food systems [3]. By leveraging their antimicrobial activity against pathogenic and spoilage microorganisms, killer yeasts or their antimicrobial subproducts can be incorporated into food production processes, either as natural additives or through fermentation-based approaches to improve food safety and quality [4]. This is in line with the growing demand for sustainable and ecological solutions, leading to innovative strategies for food preservation and pathogen control [5].

Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 and UIDB/04050/2020 units, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020 and Associate Laboratory ARNET, LA/P/0069/2020 (<https://doi.org/10.54499/LA/P/0069/2020>). D.A thank FCT for the CEEC Individual (2023.06040.CEECIND) and S. Silva for the CEEC Institutional (<https://doi.org/10.54499/CEECINST/00018/2021>).

References:

- [1] Georgescu, V. Corbu, O. Csutak, Curr. Issues Mol. Biol., 46 (2024).
- [2] S. Billerbeck, R. Walker, I. Pretorius, Trends Biotechnol., 42 (2024).
- [3] L. Said, H. Gaudreau, L. Dallaire, M. Tessier, I. Fliss, Ind. Biotechnol., 15 (2019).
- [4] M. Villalba, J. Sáez, S. Monaco, C. Lopes, M. Sangorrín, Int. J. Food Microbiol, 217 (2016).
- [5] S. Ioannidou, C. Cascio, M. Gilsenan, Environ. Int., 149 (2021).

Innovative approaches toward natural, healthier, and clean-label meat products

Ana Rita Pereira, Ana Fernandes*, Iva Fernandes, Nuno Mateus, Victor de Freitas

LAQV-REQUIMTE, Faculty of Sciences, University of Porto, Porto, Portugal

* ana.fernandes@fc.up.pt

Growing consumer demand for healthier and clean-label products has driven industries to explore natural alternatives to artificial ingredients [1]. Plants, vegetables, and agri-food co-products rich in natural pigments (e.g. anthocyanins and betalains) and bioactive compounds, such as phenolic compounds and dietary fibers, offer potential as natural colorants, fat replacers, or flavoring agents in meat products [2]. Yeast protein extract (YPE), due to its balanced amino acid profile, vitamins, antioxidants, and natural umami-enhancing properties, is a versatile ingredient that improves taste and quality [3]. This study aimed to develop meat hams with reduced salt content and vegetable-based colorants. Based on a protocol from the Primor Group, several formulations were prepared and evaluated using Texture Profile Analysis (TPA) and CIELab color attributes. The salt content in the formulation was reduced by 25% and 50% using YPE at different concentrations. The salt reduction primarily decreased the texture parameters, and the addition of YPE did not fully compensate for this loss. The 25% salt reduction showed smaller differences from the control, with lower YPE levels yielding texture values closer to the control, particularly for hardness, cohesiveness, and chewiness. Elasticity slightly increased with higher YPE levels. For a reduction of 50% of salt, a higher YPE amount leads to a lower hardness, springiness, and chewiness but higher cohesiveness. Chromatic analysis revealed no notable differences from the control, except a slight increase in the a^* parameter for higher YPE concentration, shifting towards more positive values, indicating a redder hue (Figure 1).

Two vegetable-based alternatives were explored as replacements for cochineal in ham formulations at varying concentrations. The extracts were physicochemical characterized, and key compounds, total phenolic content, and antioxidant activity were identified through DPPH and FRAP assays. Betalain-rich concentrates (BC) exhibited a concentration-dependent shift towards more reddish tones, with the a^* coordinate increasing at higher concentrations. 3% concentration showed excellent results compared to the control (produced with cochineal) with an $\Delta E \approx 6$. The anthocyanin-rich extract (AE) produced a purplish hue at higher concentrations and emerged as a promising candidate at a concentration equivalent to cochineal (0.025%) (Figure 1).

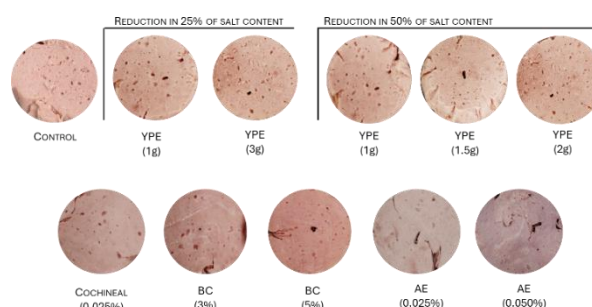


Figure 1. Meat ham formulations with reduction of salt with incorporation of yeast protein extract (YPE), and replacement of cochineal colorant by a betalain-rich concentrate (BC) and anthocyanin-rich extract (AC).

Funding: This work received financial support from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

Acknowledgments: The authors acknowledge the project VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar: PRR - C644929456-00000040 – VIIAFOOD, to Primor Group for their collaboration and support, and to Proenol for supplying Divino (YPE). AF acknowledges FCT for the research contract (CEECIND/00029/2018/CP1545/CT0010).

References:

- [1] Grand view research, Natural Food Color Market Size & Trends, 2023 - 2030.
- [2] Trincão V, Mateus N, de Freitas V, Fernandes A. Foods, (2024);13(14).
- [3] Wang H, Wang W, et al. Food Chemistry (2023);15:429:136807.

Innovation on vegan ham analogues using new protein sources

Marisa Cristo¹, Daniela Martins^{1,2}, Maria A. Azevedo³, Adriana Ferreira⁴, Cristina M. R. Rocha^{1,2}, José A. Teixeira^{1,2}, Catarina Teixeira-Guedes^{1,2,*}

¹ CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

² LBBELS – Associate Laboratory, Braga/Guimarães, Portugal

³ Colab4Food – Laboratório Colaborativo para a Inovação da Indústria Agroalimentar, Vila do Conde, Portugal

⁴ Departamento de R&D, Primor Charcutaria Prima – S.A., Vila Nova de Famalicão, Portugal

* catarina_teixeira@ceb.uminho.pt

The challenges in food production, driven by environmental and health concerns, are leading to a shift in consumer preferences towards alternative and more sustainable protein sources. The market for vegetarian and vegan meat alternatives is growing, yet market acceptance poses a challenge when talking about the appearance and sensory qualities of these formulations, as they differ greatly from traditional meat products [1,2].

To address these challenges, two fungal-derived proteins (FP1 and FP2) and one fungal-fermented protein (FFP) were used to formulate vegan ham analogues. The formulations were optimized in terms of composition (protein and lipid content), texture and appearance. A vegetarian ham containing egg white protein (EWP) served as the control for comparison.

In the first stage, all formulations were produced at a laboratory scale and later scaled up to validate the findings. At the laboratory scale, texture profile analysis revealed that FP2 achieved the best performance across all analyzed parameters, closely resembling those of EWP, followed by FFP. On the other hand, FP1 showed a significantly lower hardness compared to EWP. After scale-up, the formulation that displayed the best results in hardness was FFP, followed by FP2. The color attributes of the scaled-up analogues were evaluated using the coordinates from the CIELAB space (L^* , a^* and b^*) and calculating the color difference (ΔE^*_{ab}) between the vegan formulations (FP1, FP2 and FFP) and the vegetarian control (EWP). The greatest color difference was observed between FP1 and EWP ($\Delta E = 24.76$) while FP2 showed the best results ($\Delta E = 15.79$) followed by FFP ($\Delta E = 20.53$).

In conclusion, this study highlights the potential of fungal-derived and fungal-fermented protein sources for developing high-quality vegan meat analogues that align with consumer preferences for sustainable and sensory-appealing alternatives.

Acknowledgments: This work was financially supported by Agenda VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040), funded by the NextGeneration EU European Fund and the Portuguese Recovery and Resilience Plan (PRR). CEB and LBBELS are also supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and LA/P/0029/2020, respectively.

References:

- [1] Ahmad, M. et al. Plant-based meat alternatives: Compositional analysis, current development and challenges. *Applied Food Research* 2, 100154 (2022).
- [2] Lee, H. J., Yong, H. I., Kim, M., Choi, Y.-S. & Jo, C. Status of meat alternatives and their potential role in the future meat market — A review. *Asian-Australas J Anim Sci* 33, 1533–1543 (2020).

A Comprehensive Review of PEF Technology and its use as a Non-Conventional Pasteurization Method – A Current Day Snapshot

Pedro António^{1,2*}, Marco Alves², Maria Paula Duarte¹, Enrique Pino-Hernandez²

¹ IMETRICs, Chemistry Department, NOVA School of Science and Technology, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

² INOV.LINEA/TAGUSVALLEY - Science and Technology Park, 2200-062 Abrantes, Portugal

* paf.antonio@campus.fct.unl.pt

Pulsed Electric Field (PEF) is an innovative non-thermal technology that aligns well with current industry and consumer demands. This processing strategy relies on the application of high-intensity and short- duration (μs to ms) electric pulses to a product placed between two electrodes¹. All applications are based on the induction of the electroporation phenomena on biological material². This review aims to provide an all-encompassing snapshot of PEF (20–80 kV/cm), highlighting its potential when used alone or in combination with mild temperature (MT, 30–60 °C), as a pasteurization method for fruit beverages and purees. Current literature pinpoints PEF as a more energy-efficient and greener alternative to conventional thermal pasteurization (TT)³. Furthermore, numerous encouraging findings at laboratory- scale⁴, have reported meaningful disruption of microbial cell membranes after PEF, resulting in microbial load reductions of $\text{Log}_{10} \geq 5.0$, without compromising the quality of final product during refrigerated storage⁴. By comparing the results of previous studies on the efficacy of PEF combined with MT and purely TT, it was found that PEF can achieve a microbial load reduction similar to TT. PEF/MT treatments were also found to be most comparable to TT while being more energy-efficient and economically sustainable^{6,7,8}. However, PEF is not devoid of drawbacks since it still has several hurdles to surpass before widespread industry application, namely, the reduced number of pilot-scale studies on the inactivation of microorganisms⁵ either with PEF alone or in combination with other methods, reduced reproducibility of existing results due to poor report of process parameters⁵, and lack of regulation are the most pressing of these issues⁵. Collaborative efforts between researchers, industry stakeholders, and regulatory bodies are still required for PEF adoption to become widespread in the food industry. PEF can become a highly successful non-conventional pasteurization method, if current gaps and challenges are effectively addressed.

Acknowledgments: Pedro António thanks INOV.LINEA-TAGUSVALLEY for welcoming him during his thesis in the ambit of the Biotechnology Master program at NOVA FCT.

Funding: This work was financially supported by the project PRR—Plano de Recuperação e Resiliência and by the NextGenerationEU funds at TAGUSVALLEY, through the scope of the Agenda for Business Innovation “Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (VIIAFOOD)” (Project no. 37 AAC n.º 02/C05-i01/2022 with the application C644929456-00000040).

References:

- [1] Barbosa-Canovas, G. V., Pierson, M. D., Zhang, Q. H. & Schaffner, D. W. Pulsed electric fields. *Journal of Food Science* vol. 65 65–79 (2000).
- [2] Naliyadhara, N. *et al.* Pulsed electric field (PEF): Avant-garde extraction escalation technology in food industry. *Trends Food Sci. Technol.* 122, 238–255 (2022).
- [3] Arshad, R. N. *et al.* Pulsed electric field: A potential alternative towards a sustainable food processing. *Trends Food Sci. Technol.* 111, 43–54 (2021).
- [5] Jin, T. Z., Zhang, H. Q. Pulsed Electric Fields for Pasteurization: Food Safety and Shelf Life. In: Demirci, A., Feng, H., Krishnamurthy, K. (eds) *Food Safety Engineering. Food Engineering Series*. Springer, Cham. (2020).
- [6] Lyng, J. G., Lalor, F., Quinn, G., Pedrós-Garrido, S. Regulation of Foods Processed by Pulsed or Moderate Electric Fields (PEF or MEF). In: Raso, J., Heinz, V., Alvarez, I., Toepfl, S. (eds) *Pulsed Electric Fields Technology for the Food Industry. Food Engineering Series*. Springer, Cham. (2022).
- [7] Araújo, A., Barbosa, C., Alves, M. R., Romão, A. & Fernandes, P. Implications of pulsed electric field pre- treatment on goat milk pasteurization. *Foods* 12, 1–14 (2023).
- [8] Dias, S., Pino-Hernández, E., Gonçalves, D., Rego, D., Redondo, L., Alves, M. Challenges and opportunities for pilot scaling-up extraction of olive oil assisted by pulsed electric fields: process, product, and economic evaluation. *Applied Sciences* 14, 3638 (2024).
- [9] Pino-Hernández, E., Valério, P., Dias, S., Evangelista, M., Alves, M. Apple puree pasteurization by pulsed electric fields: from microbial safety to financial viability assessment. *ICoWEFS* 47-57 (2023)

Evaluation of the technological and microbiological characteristics of cooked smoked bacon with nitrite reduction

Maria J. M. Nunes^{1,*}, Lúcia Noronha², Inês Cruz³, Fátima Carvalho³, Paula Teixeira¹

¹Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal;

² Associação Colab4Food, Rua dos Lagidos, 4485-655, Vairão, Portugal;

³ Primor – Charcutaria Prima, S. A., Avenida de Santiago de Gavião, 4760-003 Vila Nova de Famalicão, Portugal

* *s-mjmnunes@ucp.pt*

Nitrite is a preservative commonly used in the meat industry, particularly to ensure quality and safety of the products. The chemical compound inhibits the growth of pathogenic bacteria, such as *Clostridium botulinum*, and protects against spoilage. Cooked meat products contain nitrite in its composition, which has raised concerns regarding potential health risks for consumers, especially due to the formation of nitrosamines [1]. In response, European Legislation has introduced new limits for nitrite addition, reducing the permissible quantity to 80 mg/kg of meat. The focus of this study is to assess the impact of nitrite reduction on the microbiological safety and organoleptic properties of cooked smoked bacon. The microbiological parameters include the evaluation of total viable counts, lactic acid bacteria, *Enterobacteriaceae*, *Escherichia coli* and pathogenic bacteria (*Listeria monocytogenes*, *Salmonella* spp. and spores of sulphite-reducing *Clostridium*), according to ISO Standards, during the product's shelf-life. Technological parameters, such as colour, water activity and pH, were evaluated at three sampling points: beginning, middle and end of the shelf life.

Despite the reduction of nitrite concentration to nearly half of its original value, the microbiological results indicate that the product remains stable and in compliance with the legal limits defined by the meat industry, with pathogenic bacteria were absence. Technological parameters were essential to understand the product's changes, and their potential correlation with microbiological growth.

Therefore, the new approach of reducing nitrite does not suggest safety concerns associated with the consumption of cooked smoked bacon. However, caution should be taken until further research confirms its safety.

Acknowledgments:

M. J. M. N. thanks FCT – Fundação para a Ciência e Tecnologia – for the Grant SFRH/BDANA/02204/2023. This work was supported by agenda VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o setor Agroalimentar (recuperarportugal.gov.pt). We would also like to thank the scientific collaboration under the FCT project UIDB/50016/2020. The author also would like to thank Primor – Charcutaria Prima, S.A and Associação Colab4Food – Laboratório Colaborativo para Inovação da Indústria Alimentar for the guidance, and TECMEAT – Centro de Competências do Agroalimentar para o Setor das Carnes (Vila Nova de Famalicão, Portugal) for the helping on sample characterization.

References:

[1] International Agency for Research on Cancer (IARC). Monographs (2015), volume 114.

How to communicate with adopters of insect-based foods: insights from a cross-cultural study in Portugal and the UK

Daniela Campelo¹, José Carlos Ribeiro², Pedro Sousa², Aline Silva^{1,2,3}, Guilherme Pereira⁴, Luís Baião^{1,2}, Célia Rocha^{1,2*}

¹Sense Test, Lda, Rua Zeferino Costa, 341, 4400-345 Vila Nova de Gaia, Portugal

²GreenUPorto - Sustainable Agrifood Production Research Centre / Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Campus de Vairão, Rua da Agrária 747, 4485-646 Vila do Conde, Portugal

³Department of Food and Nutritional Sciences, Harry Nursten Building, University of Reading, Whiteknights, Reading RG6 6DZ, UK

⁴Sustainable Food Products Ida, Rua Engenheiro Adelino Amaro da Costa n35 C, Esposende, 4740- 472 Braga, Portugal

**celia.rocha@sensetest.pt*

Despite a growing popularity, consumer acceptance of edible insects remains a key challenge of this sector [1,2]. One of the key strategies to improve consumer attitudes pertains the development of appealing products [3]. Among the factors involved in food product development, package design and information has been scarcely studied in insect-based food products despite its key role in influencing perceptions and possible encouraging a shift towards insect-based food products as a novel and sustainable food source [4].

The present study aimed to assess determinants of acceptance of insect-based food products and to understand how consumers valorize different communication forms of claims related to product origin (Portugal/Europe), nutritional/health/sustainability benefits, and sensory/hedonic properties. A web-based survey applying a choice-based conjoint analysis method on two insect-based products (protein bars and burgers) was performed in two European countries, Portugal and the United Kingdom. The choice design included 66 choice sets per product, with each participant evaluating 6 choices per product. Additionally, participants were characterized concerning food choices motives and behaviors, attitudes towards edible insects and sociodemographic characteristics. A total of 438 responses (Portugal, n = 208; UK, n = 230) were deemed valid after applying the validation criteria.

Portuguese consumers exhibited higher acceptance for 'snack composed by whole edible insects' and brownies/digestive biscuits with insect flour than UK consumers. Willing consumers presented high interest and experience with consuming edible insects, low food neophobia and disgust towards edible insects. Food choice motives had a lesser effect, and while interest in the healthiness and sustainability of food products had a positive effect in insects' acceptance, in the UK willing consumers were also characterized by a lower interest in the naturalness of food products. The findings from this study highlight how industry stakeholders can better reach willing consumers to increase their likelihood of accepting insect-based products.

Acknowledgments: This work has received funding from project "Great Green Minds", funded by GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável. Author J. C. Ribeiro acknowledges the PhD Researcher Contract within the "VIAFOOD" Agenda, approved in the PRR programme (Notice No. 02/C05- i01/2022) under No. C644929456-00000040, for the RTD project "HIT-C— High Integration Technology for Consumer Insights. Authors, J.C. Ribeiro, P. Sousa, A. Silva, L. Baião and C. Rocha acknowledge additional support from FCT through UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável.

References:

- [1] L. Cunha, J.C. Ribeiro, in *Edible Insects in the Food Sector: Methods, Current Applications and Perspectives* (pp. 57-71). G. Sogari, C. Mora, D. Menozzi (Eds.), Springer International, 2019.
- [2] J.C. Ribeiro, A. T. S. Gonçalves, A. P. Moura, P. Varela, L. M. Cunha, *Food Quality and Preference*, 102 (2022) 104650.
- [3] J.C. Ribeiro, M. Pintado, L. M. Cunha *Comprehensive Reviews in Food Science and Food Safety*, 23 (2024) 1.
- [4] B. Puteri, M. Oehlmann, B. Jahnke, *Food Research International*, 196 (2024) 114994.

Development of Innovative Ready To Use Industrial Pastry Preparations Using Insect Based Ingredients

Rui Rodrigues^{1,2*}, João Gomes², Ricardo Pereira^{1,2}, Miguel Azevedo³, Isabel Oliveira³ and José Teixeira^{1,2}

¹ CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

² LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

³ Decorgel – Produtos alimentares S.A., R. Progresso 363, 4785-647 Trofa

* ruirodrigues@ceb.uminho.pt

The interest in insect-based ingredients as a protein source has grown significantly in recent years due to their nutritional benefits, environmental sustainability, and potential to address global food security. Insect-based ingredients have a high protein content, are rich in micronutrients and present suitable alternative to conventional proteins¹. However, there are several challenges when incorporating these ingredients in food products, particularly in pastry applications. The functionality of insect ingredients is limited and can compromise the stability and quality of food products. They can also have a significant impact in organoleptic properties due their marked flavour, colour and textural properties².

In this study, insect ingredients were used on the development of pastry products. These ingredients were characterised by their physicochemical properties and the impact of several processing strategies were evaluated. Based on this characterization, strategies for the incorporation of insect ingredients in new pastry products were developed. The target products were cake creams and filling based on gelled emulsions and containing high protein content. The developed formulations were evaluated in terms of stability, texture, colour, water activity and organoleptic profile. These products successfully incorporated insect ingredients, can be classified as source of proteins or protein rich products and presented physicochemical and organoleptic properties compatible with pastry applications.

This study demonstrated the potential of insect ingredient in pastry applications, enabling the development of healthier and more sustainable products.

Acknowledgments: This study was founded by VIIAFOOD agenda, project supported under the PRR (www.recuperarportugal.gov.pt). This study was also supported by the Portuguese Foundation for Science and Technology(FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020.

References:

- [1] Borges MM, da Costa DV, Trombete FM, Câmara AKFI. Edible insects as a sustainable alternative to food products: an insight into quality aspects of reformulated bakery and meat products. *Curr Opin Food Sci.* 2022;46:100864. doi:10.1016/j.cofs.2022.100864
- [2] Kouřimská L, Adámková A. Nutritional and sensory quality of edible insects. *NFS J.* 2016;4:22-26. doi:<https://doi.org/10.1016/j.nfs.2016.07.001>

Colour improvement of halophyte plants for application as salt substitutes in ham products

Falcão, L. R.,^{1,*} Carvalho, L.⁴, Nunes, N. A.^{1,2}, Cruz, I.⁵, Raymundo, A.⁴, Sousa, I.⁴, Bronze, R. M.^{1,2,3}, Serra, A. T.,^{1,2} Saldanha do Carmo, C.^{1,2}

¹ iBET (Instituto de Biologia Experimental e Tecnológica), Oeiras, Portugal

² Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

³ Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

⁴ LEAF - Linking Landscape, Environment, Agriculture and Food Research Center, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

⁵ R&D Department, Primor Charcutaria-Prima, S.A., Gavião, 4760-003 Vila Nova de Famalicão

* rita.falcao@ibet.pt

Salt is widely used in the food industry as a flavouring agent, preservative, and texture enhancer. However, excessive salt intake represents a major public health concern [1]. Halophyte plants have shown potential as natural salt substitutes due to their mineral composition [2]. Nevertheless, the high chlorophyll content may affect the food's appearance, potentially reducing consumer acceptance. This study aimed to optimize a solid-liquid extraction process for chlorophyll removal from *Salicornia ramosissima* (SR) while preserving the mineral content and improving colour properties.

Response Surface Methodology (RSM) was employed to optimize the extraction process and evaluate the effect of extraction time and plant-to-solvent ratio (P:S) on extraction yield (EY), chlorophyll yield (CY), and total chlorophyll content in the extract (TC). Ethanol, a biocompatible solvent, was used at room temperature. The plant material, before and after extraction, was analysed for mineral content and colour parameters (CIELab). The optimized conditions were applied to three additional halophyte species namely, *Sarcocornia fruticosa*, *Crithmum maritimum* and *Inula chritmoides*. The resulting salt substitutes were incorporated into ham formulations. The ham products were analysed regarding texture (through texture profile analysis – TPA) and colour.

The P:S was the most significant parameter, with lower ratios increasing both CY and EY while decreasing TC, leading to brighter and less green plant material. Furthermore, longer extraction times enabled higher CY and EY of the processes and improved the colour properties of the remaining plant material. The optimized process showed consistent results across the other halophyte species and preserved the mineral content. Incorporating the developed salt substitutes into ham formulations did not significantly change the texture or colour compared to the control. This study highlights the potential of halophyte plants as a natural alternative to traditional salt, offering improved nutritional profiles without compromising food product sensory properties.

Acknowledgments: The authors acknowledge the PRR through the Agenda VIIAFOOD – Plataforma para a Valorização, Industrialização e Inovação comercial para o Agroalimentar”, framed in the notice nº 02/C05-i01/2022. The authors also acknowledge Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020).

References:

- [1] Y. Bi, L. Liang, K. Qiao, J. Luo, X. Liu, B. Sun, Y. Zhang, Food Research International, 194 (2024) 114880
- [2] M. Fitzner, M. Schreiner, S. Baldermann, Journal of Food Composition and Analysis, 122 (2023) 105435

Optimizing the extraction of elderberry anthocyanins for application as natural colorant in ham formulations

Leonor Teixeira da Costa^{1,2,*}, Ana Rita Pereira⁴, Ana N. Nunes^{1,2}, Rita Falcão^{1,2}, Maria Rosário Bronze^{1,2,3}, Inês Cruz⁵, Ana Fernandes⁴, Ana Teresa Serra^{1,2}, Cátia Saldanha do Carmo^{1,2}

¹iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901, Oeiras, Portugal

²Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

³IMED, Faculdade de Farmácia da Universidade de Lisboa, Av. das Forças Armadas, 1649-019 Lisboa, Portugal

⁴LAQV-REQUIMTE, Faculdade de Ciências da Universidade do Porto, Porto, Portugal

⁵R&D Departament, Primor Charcutaria-Prima, S.A., Gavião, 4760-003 Vila Nova de Famalicão

*leonor.costa@ibet.pt

Colour is an important organoleptic characteristic of food products, significantly influencing consumer acceptability [1]. European elderberry (*Sambucus nigra*) has been used in food, being recognised for its high anthocyanin content [3,4]. Elderberry bioactives include cyanidin derivatives, flavonols and phenolic acids [3,5,6]. Anthocyanins are already approved as food additive (E163) in the EU [2], further highlighting their potential in the food industry. This work aimed to optimise a conventional hydroalcoholic extraction process of elderberry to maximise anthocyanin yield (AY), total anthocyanin content (TAC) and extraction yield (EY), and evaluate its application as natural colourant in ham formulations.

Response Surface Methodology following a Central Composite Face-Centered design was employed, varying solid-to-solvent ratio (1:200 – 1:10), ethanol content (0-100 vol.%) and extraction time (10-60 min). Seventeen extracts were obtained and characterised for anthocyanin concentration (pH differential method and HPLC-DAD-UV), colour properties (CIELAB method) and antioxidant activity (ORAC). The extract obtained at optimal conditions was incorporated into ham formulations, and colour attributes were assessed.

Ethanol content had the most significant impact in all responses. Lower ethanol content and solid-to-solvent ratio, enhanced AY and EY. A simultaneous increase in these parameters promoted the increase in AY. Higher TAC values were obtained with an increase in the ethanol content and extraction time, independently. In this work, the EY varied between 13-54%, AY between 3-90% and TAC between 0.2-1.2g/100gd_{ext.} Optimal conditions were identified, yielding major anthocyanins, namely cyanidin-3-glucoside and cyanidin-3-sambubioside quantified by HPLC. Moreover, quercetin-3-rutinoside and quercetin-3-glucoside were also found in considerable concentrations. Minor compounds included chlorogenic, neochlorogenic acid and kaempferol-3-glucoside. Furthermore, ORAC varied between 348 and 2329 $\mu\text{mol TEAC/g}$ dried extract. An anthocyanin-based colorant was successfully produced and incorporated into ham formulations demonstrating colour properties comparable to the control colorant, with $\Delta E < 4$. A slight decrease in redness was noticed, while yellowness remained consistent across all formulations.

Acknowledgments: The authors acknowledge the PRR through the Agenda VIIAFOOD – Plataforma para a Valorização, Industrialização e Inovação comercial para o Agroalimentar”, framed in the notice nº 02/C05-i01/2022. The authors also acknowledge Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020). The authors also acknowledge FCT for the PhD grant of Leonor Costa (2021.08117.BD) and the CEEC grant of Teresa Serra (CEEICIND/04801/2017) and Ana Fernandes (CEEICIND/00029/2018/CP1545/CT0010).

References:

- E.N Veja, M. Ciudad-Mulero, V. Fernández-Ruiz, L. Barros & P. Morales. *Foods*, 12 (2023), 4102
- EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *EFSA Journal*, 11 (2013), 3145
- S. Kim, H. Son, S. Y. Pang, J. J. Yang, J. Lee, K. H. Lee, J. H. Lee, C. Park & H. Y. Yoo. *Processes*, 11 (2023), 72
- L. Ceslová, P. Kalendová, L. Dubnová, M. Pernica & J. Fischer. *Molecules*, 28 (2023), 6690
- J. Nakajima, I. Tanaka, S. Seo, M. Yamazaki and K. Saito. *Journal of Biomedicine and Biotechnology*, 5 (2004), 241–247
- P. Silva, S. Ferreira and F. M. Nunes. *Industrial Crops and Products*, 95 (2017), 227–234

Hyperbaric Inactivation – A novel nonthermal methodology to destroy *Bacillus subtilis* spores at room temperature?

Carlos A. Pinto¹, Alireza Mousakhani Ganjeh¹, Jorge A. Saraiva^{1*}

¹LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

*jorgesaraiva@ua.pt

Hyperbaric inactivation (HI) is a nonthermal processing methodology that uses moderate pressures (≤ 250 MPa) for long dwell-times (hours to days) to achieve pasteurization standards in heat and pressure-sensitive foods. Recently, this methodology has shown great potential to not only inactivate vegetative microorganisms but also bacterial spores [1], which are very resistant to conventional thermal pasteurization and high-pressure processing, being required temperatures >100 °C for several minutes to inactivate them [2]. Considering the importance of spores for both food safety and shelf-life, it is of utmost importance to evaluate the feasibility of this methodology for spore inactivation in a range of pH-values pertinent for food safety.

This work aimed evaluating the potential of HI to destroy *Bacillus subtilis* spores, and the dependence of pH and nutrient-availability for spores' inactivation, and the inherent inactivation kinetic parameters. *B. subtilis* spores were inoculated in nutrient-free Mcllvaine-buffer and Brain-heart-infusion-broth at three different pH-levels (4.50, 6.00 and 7.50) and kept under HI (150, 200 and 250 MPa) up to 7 days at uncontrolled room temperature (18-23 °C, RT).

The results demonstrated a clear dependence of nutrient-availability and pH upon spore inactivation during HI, which allowed to fit both linear and non-linear (Weibull, Log-logistic and Biphasic) kinetic models frequently used to describe spores' inactivation patterns. Lower pH-values hindered spore inactivation at 150 MPa, which could be surpassed at and above 200 MPa. The presence of nutrients accelerated spore inactivation, which impacted the inactivation kinetic parameters. Curiously, a pressure increase from 200 to 250 MPa did not accelerate spore inactivation at pH 6.00 and 7.50. Phase-contrast microscopy images revealed that the spores were inactivated without reaching the vegetative state, which is an important outcome for food safety.

These results show that HI can be an interesting approach for the inactivation of bacterial spores at RT, without applying any heat, which could be particularly interesting for heat-sensitive foods and other types of matrices.

Acknowledgments: This work received financial support from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds. Carlos A. Pinto and Alireza Mousakhani Ganjeh also acknowledges FCT/MCT for the PhD grant reference SFRH/BD/137036/2018 and 2022.12558.BD, respectively.

References:

- [1] C. Pinto, et al., Innovative Food Science & Emerging Technologies 98 (2024): 103861.
- [2] K. Reineke, et al., Annual review of food science and technology 11.1 (2020): 255-274.

Exploring the antibacterial potential of cell-free supernatants from probiotic strains against foodborne pathogens

Emílio Gomes^{1,2,3*}, Sara Ferreira^{1,2}, Daniela Araújo^{3,4}, Nuno F. Azevedo^{1,2}, Carina Almeida^{1,2,3,4}, Joana Castro^{1,2}

¹ LEPABE— Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

² ALiCE— Laboratório Associado para a Inovação em Engenharia Química, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³ Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV, I.P.), 4485-655 Vila do Conde, Portugal

⁴ Centro de Engenharia Biológica, Universidade do Minho 4710-057 Braga, Portugal

* up202008704@up.pt

Foodborne pathogens are a recurring public health issue, causing frequent outbreaks with potentially drastic consequences. To combat these microorganisms, various measures are commonly employed, including the use of antibiotics and disinfectants. However, the excessive use of these substances can lead to the development of resistance or tolerance in microorganisms. Therefore, it is important to explore new strategies that neutralize pathogenic action without exacerbating bacterial resistance to antibiotics and disinfectants. In this context, the potential of cell-free supernatants (CFSs) from organisms described as probiotics (*Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus acidilactici*, *Limosilactobacillus fermentum*, *Lactobacillus gasseri*, and *Lacticaseibacillus casei*) was evaluated against foodborne pathogens, namely *Escherichia coli*, *Salmonella enterica* (serovars Enteritidis and Typhimurium), *Listeria monocytogenes*, and *Proteus mirabilis*.

Agar well diffusion assays showed that the tested CFSs inhibited the growth of the studied microorganisms. This inhibitory activity is likely linked to the presence of organic acids, hydrogen peroxide, and bacteriocins. CFSs produced by *L. gasseri* and *L. plantarum* exhibited the largest inhibition zones. The ability of the CFSs to inhibit biofilm formation by pathogenic organisms was also assessed by quantifying biofilm biomass and cell culturability. Biofilms formed in the presence of CFS (40% v/v) had at least 94% less biomass than those without CFS. Regarding the culturability of biofilm cells, all values above zero were below the detection limit, indicating that the CFSs inhibited biofilm formation. These results highlight the action of CFSs from probiotic microorganisms against foodborne pathogens, emphasizing their potential application as alternatives to disinfectants in the food industry. Additionally, CFSs provide preventive solutions against pathogenic biofilm formation, a significant challenge in this field.

Acknowledgments: This work was funded by national funds through FCT/MCTES (PIDDAC), under the project 2022.07654.PTDC - NAMSal. This study was also supported by FCT through the strategic funding of the unit UIDB/04469/2020, UIDB/00511/2020, and UIDP/00511/2020, and ALiCE, LA/P/0045/2020. Joana Castro and Daniela Araújo thank FCT for the individual CEEC (2022.06886.CEECIND/CP1737/CT0001 and 2023.06040.CEECIND, respectively).

References:

- [1] Abdelhamid AG, Faraone JN, Evans JP, Liu SL, Yousef AE. SARS-CoV-2 and Emerging Foodborne Pathogens: Intriguing Commonalities and Obvious Differences. *Pathogens*, 2022; 11(8), 837.
- [2] Hassan A, Khan MKI, Fordos S, Hasan A, Khalid S, Naeem MZ, Usman A. Emerging Foodborne Pathogens: Challenges and Strategies for Ensuring Food Safety. *Biology and Life Sciences Forum*. 2024; 31(1):32

Hyperbaric storage as a preservation methodology to control the germination and development of *Clostridium perfringens* spores

Jorge A. Saraiva^{1*}, Carlos A. Pinto¹, Alireza Mousakhani Ganjeh¹

¹LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

*jorgesaraiva@ua.pt

Clostridium perfringens is a pathogenic bacteria associated with foodborne illness, being able to form spores that are very resistant to thermal and nonthermal pasteurization processes [1]. To temporarily delay spores' development in pasteurized foods, refrigeration is the most used strategy, yet with considerable associated energetic costs. Hyperbaric storage (HS) is a novel food preservation technique that controls storage pressure and temperature, aiming to inhibit microbial growth similarly to the conventional refrigeration processes. HS offers several advantages over refrigeration, particularly when applied at uncontrolled room temperatures (RT) [2].

This study focuses on evaluating the potential of HS/RT in inhibiting the development of the spores from *Clostridium perfringens* and comparing it with conventional refrigeration. To do so, *C. perfringens* spores were inoculated in brain-heart infusion (BHI) broth, used as a model system, and coconut water, used as a real food product for validation. Various HS conditions were tested (ranging between 75-200 MPa), for up to 30 days at uncontrolled RT. Control samples stored at atmospheric pressure and under refrigeration (4 °C) were also evaluated.

The results revealed that HS/RT effectively inhibited the germination of *C. perfringens* spores in both BHI-broth and coconut water, surpassing the performance of refrigeration. However, the efficacy of HS was found to be pH-dependent, particularly in for BHI-broth. In coconut water, HS led to a gradual reduction of the spore counts of ≈ 3 -log units after 30 days, regardless of the pressure level. Conversely, minor reductions of approximately 1-log unit were observed in BHI-broth.

In conclusion, HS/RT demonstrated the ability to prevent the development of *C. perfringens* spores in a model system (BHI-broth), being this finding validated in highly perishable coconut water. HS/RT also exhibited the potential to destroy *C. perfringens* spores during storage, particularly in coconut water, without thermal input, holding great potential for ensuring food safety.

Acknowledgments: This work received financial support from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds. Carlos A. Pinto and Alireza Mousakhani Ganjeh also acknowledges FCT/MCT for the PhD grant reference SFRH/BD/137036/2018 and 2022.12558.BD, respectively.

References:

- [1] C. Pinto et al., *Foods*, 13.12 (2023): 1832.
- [2] V. Lima, et al., *Trends in Food Science & Technology* (2023): 104126.

Emulsion-filled gels for 3D printing of customized foods

Ana C. Lima^{1,2,3*}, Tamara Calvo-Correas⁴, Arantxa Eceiza⁴, Madalena Maria Gomes de Queiroz Dias^{2,3}, Arantzazu Santamaria-Echart¹, Maria Filomena Barreiro¹

¹CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

²LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal

⁴Materials + Technologies' Group (GMT), Department of Chemical and Environmental Engineering, Faculty of Engineering of Gipuzkoa, University of the Basque Country (UPV/EHU), Plaza Europa 1, Donostia-San Sebastian, 20018, Gipuzkoa, Spain

* anacarolina.lima@ipb.pt

Soft solid systems containing dispersed fillers have attracted interest in the food industry due to their remarkable stability, tunable rheological properties, and potential to deliver lipophilic nutraceuticals [1]. Among these systems, emulsion-filled gels (EFGs), which consist of emulsion droplets dispersed in a polymeric gel matrix, combine the advantages of emulsions and hydrogels [2]. Their shear-thinning behaviour, dimensional stability, and mechanical strength make EFGs ideal for extrusion-based 3D printing [3], enabling the creation of stable structures visually appealing, texturally diverse, and nutritionally customized for individuals with swallowing difficulties, children with sensory sensitivities, or personalized dietary needs [4].

This study developed EFGs by incorporating a high internal phase emulsion (HIPE) into a hydrogel matrix, systematically varying the emulsion content from 5% to 50% by total weight to optimize the printability. The effects of system formation and gelling were evaluated through rheological tests and 3D printing after 24 and 48 hours of sample preparation.

All EFGs systems showed improved rheological performance compared to the base HIPE. Incorporating the emulsion into the gel matrix significantly increased the initial viscosity while maintaining the pseudoplastic behaviour of the base emulsion. Additionally, the gel-like properties of the systems were enhanced, including higher storage modulus and yield stress, confirming their suitability and stability for 3D printing.

Results from 3D printing indicated that samples printed after 24 hours of gelation attained higher performance with increased emulsion content. The sample containing 50% wt. emulsion showed the most pronounced gel characteristics and superior recovery capacity, resulting in structures with promising fidelity. Samples printed after 48 hours of gelation also demonstrated satisfactory printing results but presented rounded corners, aligning with their higher gel character in rheological tests. In conclusion, incorporating the HIPE into the hydrogel matrix enhanced the dimensional stability and printability of the EFGs, confirming their potential in 3D-printed food applications.

Acknowledgments: This work was financed by the Portuguese Foundation for Science and Technology (FCT), by the national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the framework of the project 2022.01308.PTDC (NutriPick3D) (DOI:10.54499/2022.01308.PTDC); FCT/MCTES (PIDDAC) support to CIMO (UIDB/00690/2020 (DOI:10.54499/UIDB/00690/2020), UIDP/00690/2020 (DOI:10.54499/UIDP/00690/2020)); SusTEC LA/P/0007/2020 (DOI:10.54499/LA/P/0007/2020); LSRE-LCM (UIDB/50020/2020 (DOI:10.54499/UIDB/50020/2020), UIDP/50020/2020 (DOI:10.54499/UIDP/50020/2020)); ALiCE LA/P/0045/2020 (DOI:10.54499/LA/P/0045/2020); IT1690-22 (GV/EJ, Spain). FCT for the PhD research grant of A. C. Lima (2021.05378.BD) and the scientific employment contract (A. Santamaria-Echart).

References:

- [1] X. Zhao, D. Li, L. jun Wang, Y. Wang, Food Hydrocolloids, 135 (2023).
- [2] X. Li, X. Chen, H. Cheng, Foods, 13 (2024) 483.
- [3] Y. Cheng, B. Wang, W. Lv, Y. Zhong, G. Li, Food Hydrocolloids, 149 (2024).
- [4] Z. Liu, M. Zhang, B. Bhandari, Y. Wang, Trends in Food Science & Technology, 69 (2017) 83.

Functionalized Plant Proteins as Stabilizers for 3D-Printable Pickering Emulsions

**Larissa Camargo Ghirro^{1,2,3}, Arantzazu Santamaria-Echart¹, Madalena M. Dias^{2,3},
Maria Filomena Barreiro^{1*}**

¹ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

² Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials (LSRE- LCM), Department of Chemical Engineering, Faculty of Engineering University of Porto, Porto, Portugal

³ Associate Laboratory in Chemical Engineering (ALiCE), Faculdade de Engenharia, Universidade do Porto, Porto, Portugal

*barreiro@ipb.pt [Maria Filomena Barreiro]

The increasing demand for personalized nutrition is fostering innovation in food technology, with 3D printing emerging as a promising approach [1]. The success of 3D food printing heavily relies on the inks' rheological and textural properties, which can be finely tuned through additives, and optimized printing conditions to enhance foods' stability and structural integrity [2]. In this context, Pickering emulsions (PEs) have shown great promise as 3D food inks, thanks to their ability to achieve tailored rheological properties by adjusting parameters like particle concentration and oil content [3]. Unlike traditional emulsions, PEs are stabilized by colloidal particles that form a rigid barrier at the oil-water (O/W) interface, providing superior stability and functionality [4].

This study explores the development of food-grade Pickering stabilizer particles derived from plant protein sources. The research aims to formulate Pickering emulsions with tailored rheological profiles, enabling their 3D printability. By leveraging the compartmentalized structure of these emulsions, the study seeks to protect and deliver combinations of active ingredients with both, similar and opposing properties within a single product. To achieve this particles, pH-shifting (pH 12) and heat treatments (70 and 90 °C) were employed to modify the protein structures, whose effects were analysed through protein solubility, particle size, zeta potential, and contact angle measurements. The results demonstrated that these treatments effectively altered the protein structures, enhancing their water affinity and improving functional properties for food applications. Subsequent steps will evaluate the Pickering emulsification capacity of the particles, followed by their application in 3D printing.

Acknowledgments: This work was supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI:10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); LSRE-LCM UIDB/50020/2020 (DOI:10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI:10.54499/UIDP/50020/2020); and ALiCE LA/P/0045/2020 (DOI:10.54499/LA/P/0045/2020). National funding by FCT, Foundation for Science and Technology, through the individual research grant of Larissa Camargo Ghirro (2022.13765.BD) and the scientific employment contract (A. Santamaria-Echart).

References:

- [1] T. Tang, M. Zhang, B. Bhandari, C. Li, Food Biosci, 59 (2024) 104112.
- [2] S. Mittal, M. Bhuiyan, M. Ngadi, Foods, 12, (2023) 4490.
- [3] Badar, Z. Wang, F. Sun, X. Xia, Q. Chen, Q. Liu, B. Kong, H. Liu, Food, 175 (2024) 113891.
- [4] L. Ghirro, S. Rezende, A. Ribeiro, N. Rodrigues, M. Carrocho, J. Pereira, L. Barros, B. Junior, M. Barreiro, A. Echart, Molecules, 27 (2022) 1250.

Hyperbaric storage does not substantially impair functional properties of egg yolk while assures microbiological safety

Gabriela Matos^{1,*}, Jéssica Tavares¹, Isabel Ferreira², Jorge A. Saraiva¹

¹LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;

²LAQV-REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto 4050-313 Porto, Portugal.

* gabrielamatos@ua.pt

Hen egg yolk is an important ingredient in a wide range of food products, due to its excellent emulsifying properties and pleasant organoleptic characteristics. According to the last EFSA reports, *Salmonella* spp., associated with the consumption of eggs and egg products, were the most frequent pairing for total number of outbreaks in EU [1].

Hyperbaric storage is a novel pressure-based food preservation methodology that uses low pressures (25-100 MPa), being a possible environmentally sustainable alternative to refrigeration since it can inhibit microbial population in many food products, at room temperature, maintaining quality properties [2]. Therefore, the aim of this work was to evaluate hyperbaric storage as a nonthermal preservation methodology on egg yolk, to assure its microbial safety and minimizing the impacts on protein structure and consequently on its functional properties.

Microbiological evaluation was assessed through counts of inoculated pathogenic *Salmonella enterica* (≈ 7.4 log CFU/mL), at different pressure levels (50/75/100 MPa), at room temperature (18-23°C), and carried out up to 60 days. Then, egg yolk was evaluated for various parameters, namely surface hydrophobicity, thermal properties through differential scanning calorimetry and emulsifying properties, during storage at 50 MPa, and compared to refrigeration.

Microbiological results showed that during hyperbaric storage the inoculated population in egg yolk, *S. enterica*, was reduced, in most cases, to counts below 1 log CFU/mL. Regarding the effect on egg yolk protein structure, at 50 MPa, the thermal denaturation enthalpy showed a progressive decrease, although without any significant change on denaturation temperature. The emulsifying activity reached its highest value (increase 1.17-fold) on day 35 under 50 MPa. Despite this, no significant changes were observed for emulsion stability and surface hydrophobicity, compared to refrigeration. This indicates that this innovative nonthermal pressure methodology has great potential to assure microbial safety, while maintaining the egg yolk properties.

Acknowledgments: Thanks are due to the University of Aveiro, University of Porto and FCT/MCTES for the financial support for the LAQV research Unit (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. Gabriela Matos also thanks FCT/MCTES and ESF through NORTE 2020 for PhD grant reference 2021.07139.BD.

References:

- [1] K. Koutsoumanis et al, EFSA Journal, 22 (2024) 1-114.
- [2] V. Lima, J. Saraiva, Trends in Food Science & Technology, 139 (2022) 104-126.

Microencapsulation of a green microalga (*Chlorella vulgaris*) for its valuation as a feasible ingredient in pear snacks

Nadjet Djihad¹, Naima Fadloun Oukil¹, Sarah Hamid¹, Amel Attia¹, Sílvia Petronilho^{2,*}

¹ Laboratoire de Biotechnologie Végétale et Ethnobotanique, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000, Bejaia, Algeria

² LAQV-REQUIMTE & CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

* silviapetronilho@ua.pt

Chlorella vulgaris is a green microalga known for its nutritional value and bioactive compounds, making it a promising functional food ingredient. However, its unpleasant taste, smell, and dark green colour, have been considered restrictive to consumer acceptance. In this study, it was hypothesized that complex coacervation could produce microcapsules to mitigate the sensory drawbacks of *C. vulgaris* in pear snacks, enhancing their acceptance and buying intention [1]. For this, two proteins with similar isoelectric points were used: the pea protein isolate (PPI) and the bovine serum albumin (BSA). Partially- sulfated carrageenan (C) was the selected polysaccharide to encapsulate *C. vulgaris* (6% w/w). The PPI/C coacervates allowed to obtain higher encapsulation yield (79%) and efficiency (75%) due to higher electrostatic interaction of the polymers at pH 3, compared to higher repulsion from some negatively charged BSA amino acids. Despite this, both microcapsules exhibited an amorphous structure and low water activity (a_w around 0.3). However, PPI/C microcapsules had significantly lower moisture and water solubility (11% and 57%, respectively), and formed continuous shells. Moreover, PPI/C microcapsules prevented the early release of *C. vulgaris* compounds at gastric conditions ensuring its later release in the intestinal tract. When incorporated into think pear snacks, the higher sensory scores and buying intention were obtained for snacks with PPI/C microcapsules containing *C. vulgaris*. Therefore, complex coacervation, particularly of PPI/C, allowed to mitigate the undesirable sensory attributes of the microalga, contributing to the future acceptance and consumption of algae- based food products.

Acknowledgments: Thanks are due to the financial support from PT national funds (FCT/MCTES) for the financial support of LAQV-REQUIMTE/University of Aveiro (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) and CICECO (LA/P/0006/2020, UIDP/50011/2020, and UIDB/50011/2020) research units and, when applicable, co- financed by the FEDER, within the PT2020 Partnership Agreement, QREN, and Compete 2020. SP thanks its research contract to the project “FAIST – Fábrica Ágil, Inteligente, Sustentável e Tecnológica” (Project no.66 with application C644917018-00000031). Thanks are also due to the mobility and financial support from Erasmus+ ICMConsortium AMIGO program and to the University of Bejaia (Algeria) for providing all the facilities necessary for the development of this work, and to SALT Services Lisbon company for their support and facilities.

References:

[1] N. Djihad, F. O. Naima, S. Hamid, A. Attia, S. Petronilho, *Algal Research* 83 (2024) 103727.

Ensuring Authenticity: Advanced qPCR Solutions for *Tenebrio molitor* and *Hermetia illucens* in Feed and Food Products

Andreia Filipa-Silva^{1*}, Thaise Martins^{1,2}, Maria J. Mota^{3,4}, André Almeida^{5,6}, Daniel Murta^{7,8}, Luísa M.P. Valente^{1,2}, Sónia Gomes^{1,2}

¹ CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal

² ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

³ SORGAL, Sociedade de Óleos e Rações, S.A., S. João de Ovar, Portugal

⁴ SAVINOR - Sociedade Avícola do Norte S.A., Trofa, Portugal

⁵ SEBOL, Comércio e Indústria do Sebo, S.A., Loures, Portugal

⁶ ITS, Indústria Transformadora de Subprodutos, S.A., Coruche, Portugal

⁷ Thunder Foods Lda., Ingredient Odyssey S.A., Santarém, Portugal

⁸ EntoGreen, Ingredient Odyssey S.A., Santarém, Portugal

* andreia.silva@ciimar.up.pt

The increasing demand for alternative protein sources has heightened the need for high throughput authentication methods for insect-based feed and food products in the agrifood sector. This study focuses on developing and validating two species-specific qPCR-based assays to detect and quantify DNA two commercially significant insect species: *Tenebrio molitor* (yellow mealworm) and *Hermetia illucens* (black soldier fly). Using primers targeting the mitochondrial cytochrome b gene for *T. molitor* and NADH dehydrogenase subunit for *H. illucens*, the assays demonstrated high sensitivity, detecting target DNA concentrations as low as 2 pg/μL for *T. molitor* and 0.2 pg/μL for *H. illucens* in complex food matrices. A key innovation is the harmonized thermal cycling conditions allowing simultaneous detection of both species in a single run, saving time and resources.

The method's robustness was validated across various commercial meals, hydrolysates, and animal feed products, accurately identifying both target species in mixtures at concentrations as low as 0.24% inclusion. No significant cross-reactivity was observed with other tested species or matrices. This robust, cost-effective approach supports regulatory compliance, ensures authenticity, and upholds safety standards in the insect-based protein supply chain, highlighting the need for advanced detection. These novel protocols set a foundation for future monitoring and quality assurance measures in food and feed industries [1].

Acknowledgments: This research was funded by the Blue Bioeconomy Pact (C644915664–00000026), for the exercise of activities in the WP6 FEED (Pep4Fish project) through international funds provided by the European Union, and supported by the strategic funding UIDB/04423/2020 and UIDP/04423/2020, and LA/P/0101/2020 (<https://doi.org/10.54499/LA/P/0101/2020>) through national funds provided by FCT – Fundação para a Ciência e a Tecnologia.

References:

[1] A. Filipa-Silva, T. Martins, M.J. Mota, A. Almeida, D. Murta, L.M.P. Valente, S. Gomes, Food Composition and Analysis, 140 (2025).

This work was developed in collaboration with industry.

Willingness to Try Insect-Based Foods in Portugal and Mexico: A Cross-Cultural Consumer Study

Rodríguez, A. M.¹, Faria, B. F.¹, Costa, A. I. A.*¹

¹CATÓLICA-LISBON School of Business & Economics, Universidade Católica Portuguesa,
Lisbon, Portugal

* anacosta@ucp.pt

Edible insects offer a sustainable solution to the growing global demand for alternative protein sources that can replace farm animal protein in human diets [1]. Insects stand out for their high nutritional value and low environmental impact [2]. Despite these benefits, acceptance of insect-based foods in Europe remains limited, due to psychological barriers like low familiarity, disgust and neophobia [1,3]. Meanwhile, eating insects is a culturally rooted practice in many non-Western countries that has been shown to promote the acceptance of insect-based foods [4].

This study investigated willingness to try insect-based foods among Portuguese and Mexican consumers to ascertain the impact of familiarity with entomophagy on acceptance of novel insect-based products and explore other cross-cultural effects. Data was collected in 2024 by administering online surveys to 121 consumers in Portugal and 158 in Mexico. Respondents were shown photos of three products – a salad with a whole insect (direct entomophagy), a shake with insect protein and a bowl of tortilla chips made with insect flour (stealth entomophagy) – and asked to rate their willingness to try them. Familiarity with entomophagy and degree of food neophobia and disgust were also assessed.

Portuguese showed lower willingness to try insect-based foods than Mexicans, irrespectively of product type. Both were more willing to try the tortilla chips than the protein shake, and this product more than the salad, suggesting that direct entomophagy is less acceptable than stealth insect integration even in countries with a tradition of this practice [1,5]. Regression analysis showed that disgust and food neophobia are strong negative predictors of willingness to try insect-based foods in both countries, but not age or education.

These results underscore the impact of cultural and psychological factors on the adoption of insect-based foods by consumers. Localized marketing strategies, educational campaigns and gender-specific approaches are essential to effectively promote these sustainable food solutions globally.

Acknowledgments: This work was financially supported by “InsectERA” (Project No. C644917393-00000032) within the WP InFood, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation.

References:

- [1] Ribeiro, J. C., Gonçalves, A. T. S., Moura, A. P., Varela, P., & Cunha, L. M. *Food Quality and Preference*, 102 (2022) 104650.
- [2] Tang, C., Yang, D., Liao, H., Sun, H., Liu, C., Wei, L., & Li, F. *Food Production Processing and Nutrition*, 1 (2019) 1.
- [3] Costa, A.I.A., Monteiro, M.J.P., Maya, C., Rocha, C. Faria, B.F., Lima, R.C., Cunha, L.M., & Roos, N., 1 (2024) 32.
- [4] Kröger, T., Dupont, J., Büsing, L., & Fiebelkorn, F. *Frontiers in Nutrition*, 8 (2022)
- [5] Tzompa-Sosa, D. A., Moruzzo, R., Mancini, S., Schouteten, J. J., Liu, A., Li, J., & Sogari, G. *PLoS ONE*, 18 (2023) 1, e0279530.

Impact of Photoselective Nets on Phenolic Compound Content in Redlum Apples

João David Teixeira^{1,2}, Miguel Leão de Sousa³, Sílvia Cruz Barros¹, Pier Parpot^{4,5}, Carina Almeida^{1,6,7}, Ana Sanches-Silva^{2,8,9}

¹ National Institute for Agrarian and Veterinary Research (INIAV), I.P., Rua dos Lágidos, Lugar da Madalena, Vila do Conde, Portugal,

² Center for Study in Animal Science (CECA), ICETA, University of Porto, Porto, Portugal

³ National Institute for Agrarian and Veterinary Research (INIAV), I.P., Alcobaça, Portugal

⁴ University of Minho, Department of Chemistry, Braga, Portugal

⁵ University of Minho, Centre of Biological Chemistry, Braga, Portugal

⁶ LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁷ ALICE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁸ University of Coimbra, Faculty of Pharmacy, Polo III, Azinhaga de Stª Comba, Coimbra, Portugal

⁹ Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal
david.teixeira@iniav.pt

Photoselective nets are a novel agricultural method for enhancing crop quality by modifying light quality and exposure [1]. First introduced in fruit crops in the 1990s, these nets act as a physical barrier and filter for sunlight, influencing factors like shading, temperature, and frost reduction [2]. This study focused on understanding how these nets affect phenolic compounds in Gala Redlum apples grown under five types of nets: Grey, Yellow, Red, Black, and White.

The phenolic compounds in apple peels, seeds, and pulp were identified and quantified using Ultra High Performance Liquid Chromatography – Time of Flight – Mass Spectrometry (UHPLC-ToF-MS) [3]. The results indicated that apples grown under photoselective nets had lower phenolic compound levels in their peels. Notably, flavanonols were absent in the control apples but present in apples from four of the five net treatments. Flavanone levels were also higher in the fruit under the nets (0.095- 0.170 µg/g) compared to the control (0.042 µg/g). The yellow and white nets notably increased flavone content, reaching 5.972 and 6.316 µg/g, respectively, compared to just 0.154 µg/g in the control.

In seed extracts, differences between control and photoselective net treatments were minimal, though the flavones, flavanones, and flavanols content was generally higher under the nets. Isoflavones were the only compound group to show higher content in fruit from net-protected orchards, particularly in the pulp.

The main phenolic compounds in the apples were quercetin in the peels (59.26 - 94.60 µg/g) and phloridzin in the seeds (68.92 - 142.36 µg/g). Despite the variation in phenolic content, no net treatment produced fruit with higher levels of phenolic compounds than the control group. This may be due to the fact that nets reduce light intensity, which lessens the environmental stress that typically boosts phenolic compound production in apples exposed to more intense sunlight and temperature fluctuations.

Acknowledgments: This work was carried out in the frame of the project IDfoods - Food System of The Future, with nr. 182848, co-financed by Operational Programme for Competitiveness and Internationalization (COMPETE 2020), through national funds. The work was supported by UIDB/00211/2020 with funding from FCT/MCTES through national funds. JDT would like to thank FCT for his fellowship (2023.04311.BDANA).

References:

- [1] T. Li, J. Zhou, R. Liu, Z. Yuan, and J. Li, "Effects of photo-selective nets and air humidity coupling on tomato resistance to *Botrytis cinerea*," *Scientia Horticulturae*, vol. 305, p. 111356, Nov. 2022.
- [2] B. S. Harish, K. Umesha, R. Venugopalan, and B. N. Maruthi Prasad, "Photo-selective nets influence physiology, growth, yield and quality of turmeric (*Curcuma longa* L.)," *Industrial Crops and Products*, vol. 186, p. 115202, Oct. 2022.
- [3] J. D. Teixeira, A. R. Soares Mateus, C. Sanchez, P. Parpot, C. Almeida, and A. Sanches Silva, "Antioxidant Capacity and Phenolics Profile of Portuguese Traditional Cultivars of Apples and Pears and Their By-Products: On the Way to Newer Applications," *Foods*, vol. 12, no. 7, p. 1537, Apr. 2023.

Around the World in 51 Octopus Dishes

Sofia Pinheiro^{1*}, Filipa Soares¹, Mariana Roriz¹, Vítor Verdelho¹

¹Cell4Food, Porto, Portugal

*sofia.pinheiro@cell4food.eu

Octopus is a highly valued delicacy across Europe, Asia, and beyond, with Europe consuming approximately 140,000 tons annually¹ and South Korea leading in per capita intake (up to 2 kg/person/year²). However, rising demand and overfishing threaten wild populations³, necessitating sustainable alternatives. Cell4Food's cellular octopus production offers an innovative solution, preserving both the culinary and cultural heritage of octopus while minimizing ecological impact.

Octopus is an exceptional source of nutrition, with a protein content of 70%–85% (dry matter) and an abundance of essential minerals like calcium and magnesium. It is also rich in vitamins A, C, and E, along with beneficial compounds such as β -carotenes and collagen⁴. This nutritional profile reinforces its global popularity, reflected in hundreds of traditional recipes worldwide.

To understand consumer preferences, we analysed the top ten nations with the highest per capita octopus consumption, identifying the five most iconic dishes from a global repertoire of 51 octopus recipes. These selections showcase the most beloved preparations across diverse culinary traditions. Three main consumption patterns emerged: whole or minimally processed octopus in simple dishes, octopus incorporated into composite preparations (e.g., fillets, cakes, pastries), and processed forms such as marinated, canned, or dried products.

At Cell4Food, we are pioneering cell-based octopus production to meet rising demand while addressing environmental and ethical concerns. By leveraging cellular agriculture, we ensure the continuity of traditional octopus cuisine without compromising marine ecosystems. Our technology-driven approach aligns with global sustainability goals, providing consumers with a responsible alternative that maintains the taste, texture, and cultural significance of octopus-based dishes.

Can you imagine enjoying a delicious "polvo à lagareiro" without any marine life being harmed in the process? That's the future we're working on!

References:

- [1] European Commission: Directorate-General for Maritime Affairs and Fisheries. (2021). *Octopus in the EU: price structure in the supply chain: focus on Italy, Spain and Greece: case study*. Publications Office. <https://data.europa.eu/doi/10.2771/87203>
- [2] Josupeit, H. (2008). World octopus market. GLOBEFISH Research Programme, Vol. 94. Food and Agriculture Organization of the United Nations
- [3] Sauer, W. H. H., Gleadall, I. G., Downey-Breidt, N., Doubleday, Z., Gillespie, G., Haimovici, M., ... Pecl, G. (2019). World Octopus Fisheries. *Reviews in Fisheries Science & Aquaculture*, 29(3), 279–429.
- [4] Almeida, C., Loubet, P., Laso, J., Nunes, M. L., & Marques, A. (2022). Environmental assessment of common octopus (*Octopus vulgaris*) from a small-scale fishery in Algarve (Portugal). *The International Journal of Life Cycle Assessment*, 27, 849–867

Effect of cherry and quince juice on the second fermentation of kombucha

Mário Cristóvão^{1*}, Rodrigo Rato^{1,2}, Luisa Paulo¹, Adriana Oliveira¹, Ana Silveira¹, Cátia Baptista¹, Ana Rodrigues¹, Ana Riscado¹, Alexandra Camelo¹, Christophe Espírito Santo^{1,3}, Inês Brandão^{1,3}

¹CATAA- Associação Centro de Apoio Tecnológico Agro Alimentar de Castelo Branco, Zona Industrial de Castelo Branco, Rua A 6000-459 Castelo Branco, Portugal, geral@cataa.pt

²UBI-Universidade da Beira Interior, Rua Marquês D'Ávila e Bolama 6201-001 Covilhã, Portugal, geral@ubi.pt

³Universidade de Coimbra, Centro de Ecologia Funcional, Departamento de Ciências da Vida, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal, cfe@uc.pt;
*mario.cristovao@cataa.pt

Consumers are increasingly interested in foods and drinks that are considered healthy, fuelling the popularity of fermented products such as kombucha on the market¹. Traditionally, kombucha is made with water, sugar, tea leaves and SCOBY (symbiotic culture of bacteria and yeast)². However, the nutritional and sensory profile of kombucha can be improved by a second fermentation using different strategies.

This work focussed on the use of cherry or quince juices for the second fermentation. The fruit juice was prepared by using of non-commercial grade fruits as a strategy to reduce food waste and minimise losses in the food chain. The juice was obtained by centrifugation, filtration (0.71 mm) and pasteurisation. This study aimed to develop a kombucha in which the first fermentation inoculation is carried out using only SCOBY, without other starters, followed by a second fermentation without sugar, which is replaced by quince or cherry juice. The final product was subjected to a nutritional analysis and hedonic test by 68 untrained consumers (56% female, 44% male).

The nutrient analysis revealed a higher carbohydrate content in the cherry kombucha (8.66g/100g) compared to the quince kombucha (4.66g/100g). Both kombuchas had an alcohol content of less than 0.5%, which categorises them as alcohol-free. Analysing the sugar content before and after the second fermentation showed a reduction in sucrose and increased fructose. The sensory evaluation confirmed that both kombuchas exceeded the acceptance threshold of 5, indicating consumer approval.

Acknowledgments: Fusilli (European Union's Horizon 2020 under grant agreement No. 101000717)

References:

- [1] Coelho, R. M. D., Almeida, A. L. de, Amaral, R. Q. G. do, Mota, R. N. da, & Sousa, P. H. M. de. (2020). Kombucha: Review. *International Journal of Gastronomy and Food Science*, 22,100272. <https://doi.org/10.1016/j.ijgfs.2020.100272>
- [2] Morales, D., Gutiérrez-Pensado, R., Bravo, F. I., & Muguerza, B. (2023). Novel kombucha
- [3] beverages with antioxidant activity based on fruits as alternative substrates. *LWT*, 189,115482. <https://doi.org/10.1016/j.lwt.2023.115482>

Innovative Valorization of Cookie By-Products for Sustainable Production of Prebiotic Fructo-Oligosaccharides

Sofia M. Amoedo¹, Rita Peixoto², José A. Teixeira^{1,3}, Clarisse Nobre^{1,3,*}

¹ Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

² Vieira de Castro, Vila Nova de Famalicão, Portugal

³ Associate Laboratory (LABBELS), Braga, Guimarães, Portugal

* clarissenobre@ceb.uminho.pt

Background: Sustainability has become a key focus for companies due to ethical responsibilities and market demand [1]. Vieira de Castro is committed to reducing waste and developing innovative solutions to valorize by-products generated during the production of cream-filled cookies. These by-products, rich in sucrose, can serve as a substrate for the synthesis of prebiotic fructo-oligosaccharides (FOS). FOS are well-established prebiotics with significant potential for modulating gut microbiota, contributing to the prevention and management of various diseases [2].

Aim: This study aimed to explore the potential of reusing by-products from cream-filled cookies as a substrate for synthesizing prebiotic FOS, contributing to a sustainable and economical valorization process.

Methods: Sucrose extraction from filled cookies was optimized using an experimental design. Subsequently, a commercial enzyme complex was utilized to enable *in situ* synthesis of FOS from the extracted sucrose.

Results: Preliminary findings demonstrated successful FOS synthesis from cookie-derived sucrose. Yields of 0.82 and 0.85 g_{FOS}/g_{initial sucrose} were achieved for two different types of filled cookies, corresponding to FOS concentrations of 44.24 and 58.41 g/L, respectively. These results are highly promising, showcasing the feasibility of efficiently converting cookie by-products into high-value prebiotic compounds.

Conclusions: By-products from cream-filled cookie production were successfully repurposed for the enzymatic synthesis of FOS on a laboratory scale, demonstrating the feasibility of using these substrates as an economical alternative to pure sucrose. This approach not only produces high-value prebiotic compounds but also aligns with the principles of sustainability by achieving zero final waste. Future work will focus on optimizing the sucrose extraction process, with results from the experimental design to be presented at the upcoming conference.

Acknowledgments: This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020, and by VIIAFOOD- A 1.9 “Development of healthier and more sustainable food products from PRR (Programa de Recuperação e Resiliência)”. Clarisse Nobre acknowledge the FCT for the Assistant Research contract (DOI 10.54499/2021.01234.CEECIND/CP1664/CT0019).

References:

- [1] D. Adams, J. Donovan, C. Topple, Business Strategy and the Environment, 32 (2023) 1451-1463.
- [2] C. Nobre, L. Simões, D. Gonçalves, P. Berni, J. Teixeira, in Current Developments in Biotechnology and Bioengineering, A. Rai, S. Singh, A. Pandey, C. Larroche, C. Soccol (Eds.), Elsevier, 2022, 109-138.

A full insight into the valorization of wheat by-products

**Daniela Marisa Ferreira^{*1}, Ezequiel Coscueta¹, Maria Emília Brassesco¹ and
Manuela Pintado¹**

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal.

* dmvferreira@ucp.pt

Wheat is one of the most consumed cereals globally, significantly contributing to human nutrition [1]. However, wheat processing generates underutilized by-products, primarily wheat germ and bran, despite their high nutritional value [2][3]. Wheat bran (WB) is a primary source of dietary fiber, proteins, and phenolic compounds and contributes to approximately 25% of the total weight of the wheat grain [4]. Therefore, studies have been conducted to valorize this by-product. Enzymatic hydrolysis combined with ultrasound-assisted extraction enhances the release of ferulic acid, phenolic compounds, and bioactive peptides, improving their antioxidant and antimicrobial properties [5]. Solid-state fermentation has further increased the bioavailability of phenolics and the solubility of dietary fiber [6]. Furthermore, this valorized WB can be used in cosmetic or food products [5][7].

Wheat germ (WG), rich in protein (34%), fat (10%), tocopherols, and essential minerals, possesses antioxidant properties [8]. This by-product can be separated in two main products, the wheat germ oil (WGO) and the wheat germ proteins (WGP). Moreover, the WGO has been effectively valorized through supercritical CO₂ extraction to obtain nanoemulsions for cosmetic and food products [9]. In addition, WGO can be extracted using cold pressing, to produce wound-healing agents [10]. Additionally, enzymatic hydrolysis of wheat germ proteins has yielded antioxidant and antimicrobial peptides [11]. These proteins and peptides can be applied in functional foods and biodegradable packaging materials [12][13]. WG proteins and peptides can also be obtained using microwave extraction for further use in wound-healing agents [14].

Despite these advances, challenges remain in scaling up extraction/valorization processes, improving economic feasibility, and achieving full valorization of wheat by-products. Future research should focus on optimizing green extraction techniques, broadening industrial applications, and promoting a circular economy in wheat processing. By integrating sustainable strategies, wheat by-products can transition from low-value waste to high-value bioactive ingredients, fostering both economic and environmental sustainability.

Acknowledgments: The authors are grateful to FEDER Cooperación Interreg VI A Espanha – Portugal (POCTEP) 2021-2027 for financial support through the project TRANSCoLAB PLUS 0112_TRANSCoLAB_PLUS_2_P.

References:

- [1] J. M. Awika, "Major Cereal Grains Production and Use around the World," 2011, Accessed: Aug. 07, 2024. [Online]. Available: <https://pubs.acs.org/sharingguidelines>
- [2] D. Si et al., "Production and characterization of functional wheat bran hydrolysate rich in reducing sugars, xylooligosaccharides and phenolic acids," *Biotechnology Reports*, vol. 27, Sep. 2020.
- [3] C. Fărcaș et al., "An Update Regarding the Bioactive Compound of Cereal By-Products: Health Benefits and Potential Applications," *MDPI*, Sep. 01, 2022.
- [4] W. Cheng, Y. Sun, M. Fan, Y. Li, L. Wang, and H. Qian, "Wheat bran, as the resource of dietary fiber: a review," *Crit Rev Food Sci Nutr*, vol. 62, no. 26, pp. 7269–7281, 2022.
- [5] Guerrini et al., "Antioxidant and antimicrobial extracts obtained from agricultural by-products: Strategies for a sustainable recovery and future perspectives," *Food and Bioprocess Processing*, vol. 124, p. 397, Nov. 2020.
- [6] N. Li et al., "Valorization of Wheat Bran by Three Fungi Solid-State Fermentation: Physicochemical Properties, Antioxidant Activity and Flavor Characteristics," *Foods*, vol. 11, no. 12, 2022.
- [7] N. T. Alzuwaid, M. Sissons, B. Laddomada, and C. M. Fellows, "Nutritional and functional properties of durum wheat bran protein concentrate," *Cereal Chem*, vol. 97, no. 2, pp. 304–315, Mar. 2020.
- [8] Khalid, A. Hameed, and M. F. Tahir, "Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality," *Frontiers Media S.A.*, 2023.
- [9] Z. P. Gumus et al., "Herbal infusions of black seed and wheat germ oil: Their chemical profiles, in vitro bio- investigations and effective formulations as Phyto-Nanoemulsions," *Colloids Surf B Biointerfaces*, vol. 133, pp. 73–80, Sep. 2015.
- [10] M. U. Nooman, H. A. Abd El-Lateaf, A. E. N. A. Khattab, M. M. Rashad, and A. S. Al-Kashef, "Exploitation of Wheat Germ for the Production of Wheat Germ Oil and Microbial Pufas and Their Potential Application as Wound Healing Agents for Human Skin Fibroblast Cell Line," *Egypt J Chem*, vol. 66, no. 10, pp. 133–143, Oct. 2023.
- [11] F. Boukid, S. Folloni, R. Ranieri, and E. Vittadini, "A compendium of wheat germ: Separation, stabilization and food applications," *Trends Food Sci Technol*, vol. 78, pp. 120–133, Aug. 2018.
- [12] S. Hosseini, M. Kadivar, H. Shekarchizadeh, M. S. Abaee, M. A. Alsharif, and M. Karevan, "Cold plasma treatment to prepare active poly(lactic acid)/ethyl cellulose film using wheat germ peptides and chitosan," *Int J Biol Macromol*, vol. 223, pp. 1420–1431, Dec. 2022.
- [13] M. Rahbari, M. Aalami, M. Kashaninejad, Y. Maghsoudlou, and S. S. A. Aghdaei, "A mixture design approach to optimizing low cholesterol mayonnaise formulation prepared with wheat germ protein isolate," *J Food Sci Technol*, vol. 52, no. 6, pp. 3383–3393, Jun. 2015.
- [14] I. Pinarli, Ş. İbanoğlu, and M. D. Öner, "Effect of storage on the selected properties of macaroni enriched with wheat germ," *J Food Eng*, vol. 64, no. 2, pp. 249–256, Sep. 2004.

Designing a healthy canned meal: chub mackerel (*Scomber colias*) and vegetables

Pais-Costa, AJ¹, Oliveira, H,^{1,2} Nunes, ML,¹ Camacho, C,¹ Marques, A,^{1,2} Augusto, HC,³ Fraqueza, MJ,⁵ Fernandes, MH,⁵ Fernandes MJ,⁵ Lima, RC,⁴ Cação, A,³ Freitas, P,³ Gonçalves, A^{1,2}

¹ CIIMAR/ CIMAR-LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto. Matosinhos, Portugal.

² IPMA, I.P. - Portuguese Institute for the Sea and Atmosphere, Division of Aquaculture, Upgrading and Bioprospection. Algés, Portugal.

³ Cofisa, S.A., Terrapleno do Porto de Pesca—Gala, Figueira da Foz, Portugal.

⁴ Sense Test - Sociedade de Estudos de Análise Sensorial a Produtos Alimentares, Lda. Vila Nova de Gaia, Portugal.

⁵ CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon. Lisboa, Portugal

*juliana.pais.costa@gmail.com

The canned fish market is expected to grow driven by the increasing demand for convenient and nutritious food options, greater awareness of the health benefits of seafood and a global shift towards sustainable food production [1]. To meet this demand, the development of canned fish-based meals can be an effective strategy. Many consumers combine canned fish with vegetables (as side dishes), which suggests that incorporating them into canned meals can provide an appealing and balanced approach for consumers. This study aimed to develop a canned fish-based meal with chub mackerel (*Scomber colias*), carrots, green beans, chickpeas, and a tomato-based filling medium. The product was produced industrially at COFISA, S.A. (Portugal), and its sterility, stability, quality, safety and acceptability were evaluated after one month of maturation, as well as after five months at room temperature and 40±2 °C (simulating 20 months of storage at 20 °C)

The results showed that, up to 20 months of storage at room temperature, (i) stability and sterility were not affected; (ii) oxidation remained low (ca. 1 mg MA/kg); (iii) minor changes in colour parameters (luminosity and chroma) did not affect the visual appearance; (iv) chemical composition was not affected; (v) pH values remained stable, with slight decreases over time and (vi) sensory analysis revealed no significant changes in taste descriptors, such as saltiness, acidity or bitterness. Consumer acceptance remained high, with more than 80% of participants rating the product between “I like it moderately” and “I like it extremely”.

These findings demonstrate that this canned fish-based meal is a strong foundation for developing nutritious, balanced meals with other fish species and seasonal vegetables. These ingredients can be selected based on price and consumer preferences, helping to meet the growing demand for convenient and healthy canned fish options.

Acknowledgments: The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) through the strategic projects UIDB/04077/2020, UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR).

Funding: This work was developed within the scope of “BLUE BIOECONOMY INNOVATION PACT” (Project N.º. C644915664-00000026) financed by NextGenerationEU, under the incentive line “Agendas for Business Innovation” of the Recovery and Resilience Plan (PRR).

References:

- [1] Canned Seafood Market Size, Share, Trends & Forecast (2025-2032).
<https://www.skyquestt.com/report/canned-seafood-market> (accessed on 30 January 2025).

Vegan cheeses - product development and physical- chemistry characteristics

Azevedo J^{1,*}, Oliveira J¹, Torres D², Correia D³, Freitas V¹ and Soares S¹

¹ FCUP- LAQV-REQUIMTE, Rua Campo Alegre, 1002, Porto, Portugal;

² FCNAUP, Rua Campo Alegre, Porto, Portugal

³ MC Shared Services S.A., Lugar do Espido, Via Norte, 4470-177 Maia, Portugal

**joana.azevedo@fc.up.pt*

The search for innovative food solutions and substitutes for animal proteins has become a priority, due to their potential to reduce environmental impact and improve human health¹. In this context, vegan cheeses stand out as promising products on the market, especially because they eliminate allergens present in cheeses of animal origin². Despite the wide range of plant-based alternatives, there is still room for significant improvements in aspects such as appearance, texture, nutritional profile and flavor³. This study investigated different sources of vegetables and grains for the development of vegan cheeses, with the main objective of creating products with physicochemical characteristics similar to those of cheeses of animal origin, but with a higher protein content. Among the main sources of vegetable protein analyzed are chickpeas, lupins and yellow peas, recognized for their high protein quality.

Different experimental processes were performed (unfermented vs. fermented; uncured vs. cured), and cheeses produced from fermented chickpeas and cured for 20 days presented satisfactory texture and flavor profiles. Parameters such as color (CieLab System), protein content (Kjeldahl method) and texture profile were compared with commercial cheeses of animal origin. First results indicated protein content of approximately 19% for chickpea cheeses, 18% for yellow pea cheeses and 28% for a combination of chickpeas and lupin, values competitive with commercial cheeses, which have 36% of protein, obtained for 1 g by dry weight. The developed vegan cheeses presented texture properties, such as hardness, cohesion, chewiness and elasticity, close to those of commercial products. While the lupin cheeses had a softer texture, the chickpea cheeses stood out due to their similarity in hardness to traditional cheeses.

Acknowledgements: Thanks for FCT/MCTES (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds.

Funding: This work was financed by PRR-Plano de Recuperação e Resiliência and by European funds Next Generation EU, under the VIIAFOOD Agenda- Aviso No 02/C05-i01/2022; Referência: PRR - C644929456-00000040 – VIIAFOOD.

References:

- [1] (1) Nežlek, J. B.; Forestell, C. A. Meat substitutes: current status, potential benefits, and remaining challenges. *Current Opinion in Food Science* 2022, 47, 100890. DOI: <https://doi.org/10.1016/j.cofs.2022.100890>.
- [2] (2) Viñas, M.; Carnés, J.; López-Matas, M. A.; Hernández, N.; Castillo, M. J.; Ibero, M. Allergy to goat and sheep cheese with tolerance to cows milk and its derivatives. *Allergologia et Immunopathologia* 2014, 42 (3), 186-190, DOI: 10.1016/j.aller.2012.08.002.
- [3] (3) Meffeh, M.; Pasqualone, A.; Caponio, F.; Faccia, M. Legumes as basic ingredients in the production of dairy-free cheese alternatives: a review. *J Sci Food Agric* 2022, 102 (1), 8-18. DOI: 10.1002/jsfa.11502 From NLM.

Olive Leaf Extract – loaded hydroxyapatite Pickering emulsions: A novel approach to mayonnaise-like food sauces

Andreia Ribeiro^{1, 2,*}, Isabel Martins^{1, 2}, Larissa C. Ghirro^{3,4}, José Carlos B. Lopes^{1,2}, M. Filomena Barreiro^{3,4}, Madalena M. Dias^{1,2}

¹LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

² ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³CIMO - Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

⁴SusTEC - Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

* asribeiro@fe.up.pt

The production of innovative food products is an emergence of the food industry to satisfy consumers' trends, namely healthy, fat-reduced, and bioactive-based functional products [1, 2]. Pickering emulsions (PEs), which are stabilised by solid particles, have been increasingly studied due to their high physical stability, and their ability to create functional and label-friendly solutions [1]. Agro-food residues are a source of bioactive molecules (phenolics, carotenoids) with potential health benefits [3]. The work aims to develop PEs stabilised with nano-hydroxyapatite (n-HAp) particles and functional PEs through the incorporation of olive leaf extract. The main goal is to create a differentiated emulsion formulation with functional and vegan characteristics as an alternative to traditional mayonnaise sauces.

This study explores the formulation of oil-in-water PEs stabilized by n-HAp and enriched with olive leaf extract. Sunflower oil served as the oil phase, while the aqueous phase contained n-HAp. Emulsions were produced using a rotor-stator homogenizer at 11,000 rpm. Olive leaf extract was obtained via supercritical CO₂ extraction (90 bar, 50 °C, 2h). The study evaluates the impact of n-HAp concentration (5-15 wt.%) and oil/water ratio (50-80% oil) on emulsion stability. The morphology, droplet size, phase inversion, rheology, and oxidative stability were also evaluated. The results indicate that the produced PEs have good stability for the tested period of 30 days, except for the 80/20 oil/water PE that separates after production. The PEs produced with higher n-HAp or oil concentrations have a semi-solid structure making them attractive options to mimic the traditional mayonnaise texture. Regarding oxidative stability, the PEs showed considerably improved stability compared to commercial mayonnaise products, which suggests higher resistance to peroxidation and a longer shelf-life for n-HAp PEs.

The n-HAp PEs can offer advantages for olive leaf extract encapsulation, providing a promising perspective to develop innovative food-like products with low-fat, eggless and functional.

Acknowledgements: This work was also supported by national funds through FCT/MCTES (PIDDAC): LSRE- LCM, UIDB/50020/2020 (DOI: 10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020); and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020); CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020) and LA SusTEC, LA/P/0007/2021 (DOI:10.54499/LA/P/0007/2020). Andreia Ribeiro acknowledges FCT for her research contract through the individual CEEC – Stimulus of Scientific Employment (DOI: 10.54499/2022.00798.CEECIND/CP1733/CT0009). The authors also thanks Fluidinova for providing the nanoXIM- HApCare paste.

References:

- [1] Ribeiro, J.C.B. Lopes, M.M. Dias, M.F. Barreiro, *Molecules*, 28 (2023) 2504.
- [2] L.C. Ghirro, S. Rezende, A. Ribeiro, N. Rodrigues, M. Carocho, J.A. Pereira, L. Barros, B. Demczuk, M.F. Barreiro, A. Santamaria-Echart, *Molecules*, 27 (2022) 1250.
- [3] E. Capanoglu, E. Nemli, F. Tomas-Barberan, *Journal of Agricultural and Food Chemistry*, 23 (2022) 6787.

Cowpea as substitute to soybean in the fermentation of miso paste: Nutritional and sensory evaluation of two varieties: 'Cara Verde' and 'Arroz'

Paula Fazenda^{1,2,3}, Luis Pinto de Andrade^{3,4}, Luisa Paulo¹, Adriana Oliveira¹, Ana Silveira¹, Cátia Baptista¹, Ana Rodrigues¹, Ana Riscado¹, Alexandra Camelo¹, Inês Brandão^{1,5}, Christophe Espírito Santo^{1,5}, Mário Cristóvão^{1*}

¹CATAA- Associação Centro de Apoio Tecnológico Agro Alimentar de Castelo Branco, Zona Industrial de Castelo Branco, Rua A 6000-459 Castelo Branco, Portugal, geral@cataa.pt

²Inovcluster- Associação do Cluster Agro-Industrial do Centro, Zona Industrial de Castelo Branco, Rua A 6000-459 Castelo Branco, Portugal, geral@inovcluster.pt;

³Escola Superior Agrária do Instituto Politécnico de Castelo Branco, Qt.ª da Sr.ª de Mércules, 6001-909 Castelo Branco, Portugal, esa@ipcb.pt;

⁴CERNAS-IPCB (project UIDB/00681/2020 funding by FCT), Castelo Branco, 6001-909 Castelo Branco, Portugal.

⁵Universidade de Coimbra, Centro de Ecologia Funcional, Departamento de Ciências da Vida, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal, cfe@uc.pt;

*mario.cristovao@cataa.pt

Miso is a traditional Japanese paste produced by fermenting soybeans using koji inoculated with *Aspergillus oryzae*¹. This study aimed to develop a soybean-free miso paste by replacing soybeans with two drought-resistant cowpea varieties² endogenous to the Castelo Branco region, 'Cara Verde' and 'Arroz'. These cowpeas were selected to improve the sustainability and nutritional profile of the product. To this end, a rice-based miso that consists of 50% koji rice was produced, and only using the remaining water from the cooked beans as a water source.

Two different methods were used for each type of bean. The first was based on commercial rice koji, while the second involved the inoculation of rice (long-grain rice) with *Aspergillus oryzae*. Boiled bean mash was added to the koji, which was then anaerobically fermented at 25±1 °C for 28 days. The samples were nutritionally characterized and subjected to a sensory panel of 30 untrained consumers (70% female and 30% male), and compared to commercial miso on the market using a hedonic test.

From a nutritional point of view, the values obtained show that both methods and bean varieties produce a product that can be considered low energy value, a low lipid content and lower salt content than the found in commercial miso. Sensorial analysis showed that the miso samples fermented from commercial koji have a lower acceptability than those fermented with inoculated long-grain rice. Between the bean varieties, the "Arroz" acceptability was the most similar to that of the control sample. Miso's sensorial profile makes it a promising ingredient for seafood-based dishes, enhancing their flavour while also meeting the current trend towards locally sourced ingredients.

Acknowledgments: Hub4food INTERREG ATLANTIC, co-funded by the European Union. EAPA_0024/2022

References:

- [1] Kusumoto K-I, Yamagata Y, Tazawa R, Kitagawa M, Kato T, Isobe K, Kashiwagi Y. Japanese Traditional Miso and Koji Making. *Journal of Fungi*. 2021; 7(7):579. <https://doi.org/10.3390/jof7070579>
- [2] Carvalho, M., I. Castro, M. Matos, T. Lino-Neto, V. Silva, E. Rosa, and V. Carnide. 2016. Caracterização agro-morfológica de acessos de feijão-frade (*Vigna unguiculata*): bases para o melhoramento. *Revista de Ciências Agrárias*. 39:506–517. doi:10.19084/rca16091.

A question of generation? – A comparative study of microbiota composition in subsequent generations of SCOBY and kombucha

Alexandra Camelo¹, Mário Cristóvão¹, Rodrigo Rato², Christophe Espírito Santo^{1,3}, Inês Brandão^{1,3}

¹CATAA- Associação Centro de Apoio Tecnológico Agro Alimentar de Castelo Branco, Zona Industrial de Castelo Branco, Rua A 6000-459 Castelo Branco, Portugal, geral@cataa.pt

²UBI-Universidade da Beira Interior, Rua Marquês D'Ávila e Bolama 6201-001 Covilhã, Portugal, geral@ubi.pt

³Universidade de Coimbra, Centro de Ecologia Funcional, Departamento de Ciências da Vida, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal, cfe@uc.pt;

* alexandra.camelo@cataa.pt

Kombucha is a non-alcoholic acidic beverage that has become increasingly popular in recent years due to its fresh flavour and many potential health benefits¹. This beverage is made by fermenting sugar and tea (*Camellia sinensis*) with a symbiotic culture of bacteria and yeast (SCOBY). SCOBY takes the form of a solid-phase cellulosic pellicle, serially transferred from a finished batch of Kombucha to a new batch of high sugar tea. A second- SCOBY is therefore originated at the end of which fermentation.

This study analysed and compared different generations of SCOBY's and Kombuchas to characterize and understand differences in microbiota composition over different generations. For this purpose, DNA was extracted from different samples of SCOBY and Kombucha and a metabarcoding assay by next generation sequencing (Oxford nanopore Technologies) was performed in order to evaluate the community of microorganisms present in each SCOBYs and the resulting kombuchas.

The results analysed by the Epi2me bioinformatic tool showed a solid presence of acetic acid bacteria, namely of the genus *Komagataeibacter* (formerly *Gluconacetobacter*), with some differences in distribution proportions between samples and analysed generations.

This study is extremely relevant because it allows to characterize and evaluate the microbial stability of the SCOBY, and subsequent kombucha, over generations and define the community of microorganisms that are key to the success of this beverage.

Acknowledgments: Fusilli (European Union's Horizon 2020 under grant agreement No. 101000717)

References:

- [1] D. Laavanya, Shivanand Shirkole, P. Balasubramanian Current challenges, applications and future perspectives of SCOBY cellulose of Kombucha fermentation, Journal of Cleaner Production, Volume 295, 2021
- [2] H. Antolak, D. Piechota., A. Kucharska. Kombucha tea—A double power of bioactive compounds from tea and symbiotic culture of bacteria and yeasts (SCOBY), Antioxidants, 10(2021). 1541.

Unravel the impact of emerging parasites in wild fish and their risk in the Agrifood Chain

Carla Sousa^{1,2}, Tatiana Afonso¹, Luísa M.P. Valente^{1,2}, Maria João Santos^{1,3}, Fernando Atroch^{1,3}, Luís Filipe Rangel¹, Camilo Ayra-Pardo¹, Rita Barracosa⁴, Ana Rita Ribeiro⁵, Maria Leonor Nunes¹, Amparo Gonçalves^{1,6}, Bruna Nunes¹, Helena Oliveira^{1,6}, Carolina Camacho^{1,6}, Paula Ramos^{1,6}, António Marques^{1,6}, Andreia Filipa-Silva¹, Sónia Gomes^{1,2*}

¹CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal

²ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

³CIIMAR, Biology Department, Faculty of Sciences of University of Porto, Porto, Portugal

⁴MC Shared Services S.A, Matosinhos, Portugal

⁵Collaborative Laboratory for the Blue BioEconomy (B2E), Porto, Portugal

⁶IPMA, IP – Instituto Português do Mar e da Atmosfera, Algés, Portugal

* sagomes@icbas.up.pt

The increasing presence of parasites in commercially important fish species raises significant concerns about fish quality, safety, and public health risks, for both consumers and economic stakeholders. The European Food Safety Authority (EFSA) identifies *Anisakis* and other helminths as major concerns in seafood safety and serious public health hazards, emphasizing the need to study emerging zoonotic diseases. However, we should not discard other pathogenic agents, such as microsporidia parasites, that may eventually pose safety or quality problems for the fish. In response to this, the Vertical FISH project focuses on understanding the impact of parasitic infections on fillet quality in three commercially valuable species, pouting (*Trisopterus luscus*), European hake (*Merluccius merluccius*) and black scabbardfish (*Aphanopus carbo*). Fresh samples, each comprising 30 specimens with viscera intact, were caught seasonally during one year from different geographical areas. We conducted comprehensive analyses including morphometric measurements, macroscopic and microscopic parasite screening, and assessment of nutritional value and organoleptic traits. Our data revealed crucial interactions between environmental factors and parasite infections, with direct implications for product marketability. These findings enable the development of novel biomarkers for quality assessment, providing the food industry with practical tools for quality control, shelf-life prediction, and supply chain management. Through this integrated approach, Vertical FISH aligns with EFSA's priorities and demonstrates how scientific innovation addresses real-world challenges in the agrifood sector, fostering competitiveness through improved product quality and safety.

Acknowledgments: This work was funded by Innovation Pact, Project No. C644915664-00000026 WP7-FISH, known as the “Blue Bioeconomy Pact”, by FCT in the framework of the Programme Portugal2020 to CIIMAR (UIDB/Multi/04423/2021 and UIDB/Multi/04423/2022), and through the grant awarded to Luís Rangel (CEECIND/03501/2017/CP1420/CT0010), Carla Sousa (2023.00570.BDANA), and Fernando Atroch (2022_105_BI_BioeconomiaAzul).

This work was developed in collaboration with industry.

Reimagining Waste: Exploring the Nutritional and Biological Power of a Freeze-Dried Snack based on Broccoli Stalk Powder

Inês Soares^{1,*}, Rita Vedor¹, Telma Orvalho², Sara Dias², Marco Alves², Daniela Machado¹, Ana Maria Gomes¹

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

² Tagus Valley – Parque Tecnológico do Vale do Tejo, 2200-062 Alferrarede – Abrantes, Portugal

*icsoares@ucp.pt

The global food industry is progressively adopting closed-loop systems to promote sustainability and resource efficiency [1]. Upcycling of food by-products has emerged as a critical strategy within this paradigm, with the aim of minimising waste while maximising the use of nutrient-rich residues [2]. Broccoli stalks, which are frequently disposed of during processing, are a valuable source of dietary fibre and bioactive compounds, making them an ideal candidate for upcycling [3].

The present study explores the incorporation of broccoli stalk powder into a novel snack formulation and evaluates its nutritional composition (AOAC procedures) and bioactive properties, including total phenolic content (measured by Folin–Ciocalteu colorimetric method), antioxidant activity (ABTS assay) and antidiabetic potential (α -glucosidase inhibition assay).

The freeze-dried snack, formulated with 30-40% broccoli stalk powder, exhibited a total phenolic content of 2 mg gallic acid equivalents (GAE)/g, an antioxidant capacity of 6 μ mol Trolox equivalents/g, and demonstrated noteworthy antidiabetic potential, exhibiting an inhibition of α -glucosidase activity of 74%. Nutritional analysis further highlights the multifunctional benefits of the developed product. Notably, the product is a substantial source of protein (24 g/100 g) and fibre (34 g/100 g), aligning with global trends towards functional, health-promoting foods. The product is also low in fat (7 g/100 g) and sugars (9 g/100 g), with a total carbohydrate content of 51 g/100 g and an ash content of 14 g/100 g.

The combination of all these characteristics underscores the multifunctionality of the snack. The incorporation of broccoli stalk powder into food formulations represents a strategy that is congruent with sustainability objectives, given its capacity to reduce food waste. This approach is consistent with the growing consumer demand for nutrient-enriched products and demonstrates the dual role of upcycled ingredients in promoting both nutritional innovation and environmental sustainability in the food industry.

Acknowledgments: We would like to thank the scientific collaboration of CBQF (UIDB/50016/2020). Rita Vedor acknowledges to Fundação para a Ciência e Tecnologia by a PhD Grant (2023.00447.BD). This work was developed under the framework of project Agenda VIIAFOOD — Platform for Valorisation, Industrialization and Commercial Innovation for the Agri-Food Industry”, financed through the Program for Recovery and Resilience (PRR). The authors would like to acknowledge Tagus Valley for their support in carrying out the project.

References:

- [1] J. Fanzo, C. Rudie, I. Sigman, S. Grinspoon, T. G. Benton, M. E. Brown, N. Covic, K. Fitch, C. D. Golden, D. Grace, M. F. Hivert, P. Huybers, L. M. Jaacks, W. A. Masters, N. Nisbett, R. A. Richardson, C. R. Singleton, P. Webb, W. C. Willett, *The American Journal of Clinical Nutrition*, 115 (2022) 18-33. <https://doi.org/10.1093/ajcn/nqab315>.
- [2] M. Thorsen, S. Skeaff, F. Goodman-Smith, B. Thong, P. Bremer, M. Miroso, *Frontiers in Nutrition*, 9 (2022) 1071829. <https://doi.org/10.3389/fnut.2022.1071829>.
- [3] V. Gómez, R. González-Barrio, N. Baenas, D. A. Moreno, M. J. Periago, *International Journal of Molecular Sciences*, 23 (2022) 13309. <https://doi.org/10.3390/ijms232113309>.

Sensory Characterization of *Hericium erinaceus*: A Lexicon-Based Approach

Mafalda Silva^{1,2}, Ana Cristina Ramos^{1,3}, Fernando H. Reboredo³, Elsa M. Gonçalves^{1,3*}

¹ Instituto Nacional de Investigação Agrária e Veterinária, Oeiras, Portugal

² Faculdade de Ciências e Tecnologias, Universidade NOVA de Lisboa (UNL), Caparica, Portugal

³ GeoBiotec—GeoBioTec Research Institute, Faculdade de Ciências e Tecnologias (UNL), Caparica, Portugal

* elsa.goncalves@iniav.pt

Mushrooms are valued for their unique flavours, textures, and health benefits. However, they are highly perishable, even under refrigeration. The flavour compounds of various mushroom species, both fresh and processed, have been extensively studied. Descriptive sensory analysis is a widely used method for identifying and quantifying the sensory attributes and intensities of food products [1].

Hericium erinaceus (*He*), commonly known as Lion's Mane mushroom, has gained increasing recognition in gastronomy and nutraceutical research. However, despite its rising popularity, the sensory characteristics remain largely underexplored. This study aims to develop a sensory lexicon for *He* fresh and after storage (3 days; 5 °C) providing a structured approach to characterizing its sensory attributes over time [2].

The study was conducted in the sensory analysis laboratory of INIAV-UTI (Oeiras, Portugal), using individual tasting booths compliant with ISO 8589:2007 standards [3]. Sensory evaluations were carried out by a trained panel of six assessors (age between: 26-62; 67% male, 33% female). Analysis of variance (ANOVA) was performed using StatisticaTM v.8 software from Statsoft. Means were separated at the 5% significance level by Tukey's HSD test. Principal Component Analysis with mapping was conducted using the mean values of each sample. Panelists evaluated 20 sensory attributes including aromas, flavours, and mouthfeel, using an adapted methodology from Chun *et al.* [4]. Intensities were rated on a hedonic scale from 0 (extremely weak) to 5 (extremely strong).

Sensory attributes exhibited some variation over storage time. On day 0, earthy, mold, bitter, and humidity had the highest intensity values. After 3 days of storage, slight increases were observed in mold, umami, and sour attributes.

This study successfully developed a sensory lexicon for fresh *He*, providing a valuable tool for characterizing its sensory profile. Furthermore, the lexicon offers insights into the quality and evolution of sensory properties during post-harvest storage.

Acknowledgments: The authors thank the sensory analysis panelists at INIAV in Oeiras.

References:

- [1] Suwonsichon, S. The importance of sensory lexicons for research and development of food products, *Foods* 2019, 8, 27. doi: 10.3390/foods8010027
- [2] Silva, M.; Vida, M.; Ramos, A. C.; Lidon, F. J.; Reboredo, F. H.; Gonçalves, E. M. Storage temperature effect on quality and shelf-life of *Hericium erinaceus* mushroom. *Horticulturae*, 2025, 11, 158. <https://doi.org/10.3390/horticulturae11020158>
- [3] ISO 8589; Sensory Analysis—General Guidance for the Design of Test Rooms. International Organization for Standardization; Geneva, Switzerland: 2007.
- [4] Chun, S.; Chambers, E., IV; Han, I. Development of a Sensory Flavor Lexicon for Mushrooms and Subsequent Characterization of Fresh and Dried Mushrooms. *Foods* 2020, 9, 980. <https://doi.org/10.3390/foods9080980>

Exploring nutritional enhancement of a ready-to-eat spinach-mash with algae

**Joana Cristina Barbosa^{1,*}, Rita Barracosa², Daniela Correia², Beatriz Grilo³,
Verónica Pedroso³, Ana Maria Gomes¹**

¹Centre for Biotechnology and Fine Chemistry, Faculty of Biotechnology, Universidade Católica Portuguesa, Porto, Portugal

² MC Shared Services S.A., Rua João Mendonça, 529, 4464-501 Senhora da Hora, Matosinhos, Portugal

³ Faster-Produtos Alimentares Lda., Zona Industrial Alto do Padrão, Lote 46, 3200-133 Lousã, Coimbra, Portugal

*jcbarbosa@ucp.pt

Consumers are increasingly open to incorporating new foods in their diet, especially if they are considered healthy and sustainable. The global demand for algae-based foods has been rising, and algae are being increasingly consumed for both their nutritional and health benefits as well as their technological advantages. In recent years, substantial evidence has been gathered to support the health benefits of algae-derived food products[1]. This promising alternative and sustainable protein source offers several advantages over other raw materials currently in use, particularly from an environmental perspective. Moreover, a key characteristic of algae is their production of micronutrients and bioactive compounds with potential benefits for promoting better human health [1].

This project aims to reformulate the original conventional recipe for *esparregado* (a traditional Portuguese mash) made from spinach, which served as a base for incorporating algae and/or microalgae. Three new formulations were developed and proposed: (1) containing 10% (w/w) *Ulva rigida*; (2) containing 10% (w/w) *Chlorella vulgaris*; and (3) combining 10% *Ulva rigida* with 6% *Chlorella vulgaris*. These formulations were produced in a semi-industrial scale by an industrial partner.

The prototypes underwent sensory and nutritional analyses to validate the claims, which aim to position the products as potential sources of protein and fibre, as well as vitamin B12 and various minerals. Thus, prototype (1) presents high fibre, vitamin B12, Magnesium, Iodine and Iron contents; prototype (2) presents high protein and zinc contents; prototype (3) claims the benefits of both. A preliminary evaluation performed by a trained panel revealed that algae addition does not negatively affect organoleptic characteristics.

In conclusion, algae proved to be a valuable addition to increase the nutritional value of a traditional food, sustainably, and without negatively impacting the traditional organoleptic properties, that consumers will certainly value, with the combination of micro- and macroalgae presenting the most attractive solution.

Acknowledgments: Special thanks are due to the industrial partner Faster – Produtos Alimentares Lda (Lousã, Portugal), for producing the formulations prototypes. This work was supported by Agendas Mobilizadoras para a Inovação Empresarial - PRR I Aviso No 02/C05-i01/2022 project VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040), WP3 – A3.6. Fostering local sea ingredients in the food value chain.

References:

[1] P. Nova, A. Martins, C. Teixeira, H. Abreu, J. Silva, A. Silva, A. Freitas, A. Gomes, Journal of Applied Phycology, 32(3) (2020) 1789-1802

Quantifying adulteration in game meat products: Is it possible?

Rita Biltes¹, Caterina Villa¹, Joana Costa¹, Isabel Mafra^{1,*}

¹REQUIMTE/LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

* isabel.mafra@ff.up.pt

Concerns about meat product adulteration are rising globally due to potential economic fraud, violation of ethical or religious dietary restrictions, and even health risks associated with consuming undeclared species. Red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) meats, among other game meats, are highly regarded as exclusive gourmet foods due to their rarity in the conventional meat market. Both game meats reach premium prices, being susceptible of adulteration due to their substitution with inferior species or the undisclosed presence of certain species. To address the growing concerns about food authenticity, DNA-based methods have become the preferred analytical approaches for species detection/quantification in animal products due to their accuracy and reliability. In the present work, novel real-time PCR methods are proposed targeting both species, validated and applied to quantify adulterations in gourmet foods [1,2].

For each species, two real-time PCR calibration models, specifically designed for raw/mildly processed or severely treated samples in complex food matrices, were proposed with acceptable performance parameters, achieving absolute and relative limits of detection of 0.01/0.04 ng of DNA and 0.5/0.05% of red/roe deer meat in raw/processed pâté, respectively, covering a dynamic range of 0.5-50%. The approaches were successfully validated and applied to 46 market samples from four European origins (Poland, Portugal, France, and Spain). The analysis revealed significant discrepancies between declared red deer and roe deer contents and actual labelled levels in all products. The global analysis of results identified 61% of mislabelled/adulterated samples due to the absence of deer species, substitution of roe deer with red deer, substitution of fallow deer with other deer species and red deer with pork, and undeclared addition of roe deer. This study demonstrates the effectiveness of the methods to quantify game meats in processed/complex foods and their usefulness as tools for routine food inspection to ensure labelling compliance.

Acknowledgments: This work received financial support from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020). C. Villa, J. Costa and I. Mafra thank FCT for funding through the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03583.CEECIND/CP1662/CT0012 DOI 10.54499/2021.03583.CEECIND/CP1662/CT0012 and 2021.03670.CEECIND/CP1662/CT0011 DOI 10.54499/2021.03670.CEECIND/CP1662/CT0011, respectively).

References:

- [1] B. M. Adenuga, R. Biltes, C. Villa, J. Costa, A. Szychaj, M. Montowska, I. Mafra. Food Control, 168 (2025) 110872.
- [2] B. M. Adenuga, R. Biltes, C. Villa, J. Costa, A. Szychaj, M. Montowska, I. Mafra. Foods, 13 (2024) 3728.

Ultra-high-temperature ohmic heating of fruit pulps

Ricardo S. Pereira¹, José A. Texeira^{1,2} and Ricardo N. Pereira^{1,2}

¹CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

²LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

* *rpereira@ceb.uminho.pt*

Ohmic heating (OH) is a sustainable innovation in food processing, generating heat internally through electrical resistance. It eliminates the need for boilers, reducing water and energy consumption, while its high efficiency minimizes waste and supports renewable energy integration. By avoiding stirring, scraping, and high thermal loads, OH reduces fouling, preserves product quality, and offers an eco- friendly, efficient solution for the food industry [1]. A significant knowledge gap exists regarding the feasibility and impact of ohmic heating at ultra-high temperatures, particularly in its application for the sterilization of food products. The objective of this study is to evaluate the feasibility of applying ultra-high temperatures (up to 150 °C) to fruit pulps using the ohmic heating effect. Specifically, it aims to determine whether any discontinuities occur in the temperature increase during the ohmic heating process.

According to the industrial supplier (SUMOL+COMPAL, S.A.), the apple and pear pulps were obtained through a solid-liquid separation process applied to the puree of these fruits. Moderate electric fields of 2 to 12 V/cm at high frequency (20 kHz) were applied to the fruit pulps. It was possible to reach temperatures above 140 °C in both pulps. To achieve a temperature differential of 120 °C, approximately 7 minutes were required. The electrical conductivity of both pulps ranged between 2 and 7 mS/cm. This range is within the applicability of the technology, which is typically limited by values below 0.3 mS/cm. In both pulps, a slight caramelization was observed due to the thermal effect and the high temperatures reached.

The results show that fruit pulps can undergo ohmic heating up to 140 °C without limitations, making it a strategic process for ensuring microbiological safety, extending shelf life, and preserving sensory and nutritional qualities through efficient direct heating.

Acknowledgments: VIIAFOOD - Platform for Valorization, Industrialization, and Commercial Innovation for the Agri-Food Sector" (Project No. 37 with application No. C644929456-00000040), funded by the Recovery and Resilience Plan (PRR) and the European Next Generation EU funds. SUMOL+COMPAL, S.A. for providing the fruit samples.

References:

[1] L. Shao, Y. Zhao, B. Zou, X. Li, R. Dai, Trends in Food Science & Technology, 118 (2021)



Valorisation of post-harvest *Solanum melongena* plant biomass as a source of bioactive ingredients for yogurt fortification

Mikel Añibarro-Ortega^{1,2}, Maria Inês Dias¹, Jovana Petrović³, Sonia Núñez^{4,5},
Marina Soković³, Víctor López^{4,5}, Lillian Barros¹, José Pinela^{1,6,*}

¹ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

² Nutrition and Bromatology Group, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E-32004 Ourense, Spain

³ Institute for Biological Research “Siniša Stanković”, University of Belgrade, National Institute of Republic of Serbia, Bulevar despota Stefana 142, 11108 Belgrade, Serbia

⁴ Instituto Agroalimentario de Aragón, IA2, Universidad de Zaragoza-CITA, 50830 Zaragoza, Spain

⁵ Department of Pharmacy, Faculty of Health Sciences, Universidad San Jorge, Villanueva de Gállego, 50830 Zaragoza, Spain

⁶ National Institute for Agricultural and Veterinary Research (INIAV), I.P., Rua dos Lágidos, Lugar da Madalena, 4485-655 Vairão, Vila do Conde, Portugal

* jose.pinela@iniav.pt

Yogurt is a widely consumed dairy product that has been enriched with vitamins, minerals, and natural extracts, among other substances. However, the addition of non-primary ingredients can influence its physicochemical properties and stability. This study investigated the phenolic composition and bioactive properties of an extract derived from eggplant (*Solanum melongena* L.) crop remains, as well as its potential for yogurt fortification. The extract was prepared and its phenolic profile characterised using HPLC-DAD-ESI/MS [1]. The antioxidant, antidiabetic, and anti-obesity properties were assessed using *in vitro* cell-based and enzyme inhibition assays, and the antimicrobial activity was tested against foodborne fungi and bacteria [1]. The extract was then incorporated into yogurt prepared with natural yogurt and skim milk [2,3]. A control yogurt without extract was also prepared. The yogurts were stored at 4 °C and analysed over 21 days for soluble sugars, organic acids, pH, titratable acidity, colour, texture, syneresis, and antioxidant activity. The extract predominantly comprised chlorogenic acid derivatives (53% of the phenolic fraction), followed by *O*-glycosylated kaempferol and quercetin. It exhibited antioxidant activity and a remarkable α -glucosidase inhibitory capacity, surprising that of the reference drug acarbose. In addition, it inhibited the formation of advanced glycation end-products, suggesting its potential for mitigating diabetes-related complications. Modest pancreatic lipase inhibition and potent antimicrobial activity against foodborne pathogens were also observed. The incorporation of the extract influenced the yogurt's pH, titratable acidity, and colour, enhancing yogurt cohesiveness during storage. Fortification also resulted in higher glucose concentrations and reduced lactic acid production, while the caffeoylquinic acids remained stable. Furthermore, yogurt fortification enhanced antioxidant activity, although this effect diminished over time. These findings suggest that eggplant by-products represent a sustainable source of bioactive ingredients suitable for yogurt fortification. Further research is needed to evaluate consumer acceptance and the potential health benefits of this fortified food.

Acknowledgments: Work supported by FCT/MCTES (PIDDAC): CIMO (UIDB/00690/2020, UIDP/00690/2020), SusTEC (LA/P/0007/2020), M.A.O. (10.54499/2020.06297.BD), M.I.D. (10.54499/CEECINST/00016/2018/CP 1505/CT0004) and L.B. (10.54499/CEECINST/00107/2021/CP2793/CT0002). J.P. and M.S. thank the Ministry of Science, Technological Development and Innovation (contract 451–03–9/2021–14/200007). V.L. thanks the San Jorge University (Proyecto Interno ID 2223025) and the Government of Aragon (Phyto-Pharm group, ref. B44_20R). To FEDER Cooperación Interreg VI A Espanha—Portugal (POCTEP) 2021–2027 (project 0033_NET4FOOD_1_P).

References:

- [1] M. Añibarro-Ortega, V. López, S. Núñez, et al., Surname, Journal name, 12 (2019) 123.
- [2] X. Chen, Z. He, L. He, et al., LWT, 173 (2023) 114383.
- [3] S.M. Ferreira, L. Santos, Food Bioscience, 51 (2023) 102293.

Vibrational spectroscopy applied to distinguish between Honey PDO Regions

Soraia I. Pedro¹, Ana Ferreira², Fátima Peres³, Eugenia Gallardo², Ofélia Anjos^{1,4*}

¹ CERNAS-IPCB - Research Centre for Natural Resources, Environment and Society, Polytechnic University of Castelo Branco, 6000-084 Castelo Branco, Portugal; soraia.pedro@ipcb.pt; ofelia@ipcb.pt

² RISE-Health, Department of Medical Sciences, Faculty of Health Sciences, University of Beira Interior, 6200-506, Covilhã; carol.sf2000@gmail.com; egallardo@fcsaude.ubi.pt

³ IPCB - Polytechnic University of Castelo Branco, 6000-084 Castelo Branco, Portugal; fperes@ipcb.pt;

⁴CBP-BI - Chromatography and Spectroscopic laboratory, 6000-084 Castelo Branco
* ofelia@ipcb.pt

Honey is a natural product produced by bees from flower nectar and is valued for its nutritional, medicinal and sensory properties, as well as its applications in food, cosmetics and medicine. Its unique composition-shaped by geographical origin and environmental conditions-reflects the specific characteristics of the production area. In recent years, Protected Designation of Origin (PDO) certification has played a crucial role in promoting regional honeys by ensuring authenticity, quality, and a clear connection to a specific production area. This certification not only protects local traditions but also enhances competitiveness in the global market.

This study evaluated vibrational spectroscopy techniques to determine the most effective method for distinguishing honeys from different PDO regions. Samples from multiple locations within the Terra Quente, Parque Natural do Montesinho, and Ribatejo Norte PDO regions were analysed using three techniques: Fourier transform Raman spectroscopy (FT-Raman), Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), and Fourier transform near-infrared spectroscopy (FT-NIR).

The analytical parameters of the honeys met the criteria required for PDO certification. The spectroscopic techniques effectively identified differences in the chemical composition of the honeys, thereby contributing to the traceability and authenticity of PDO-certified products. Among the methods tested, FT-Raman proved to be the most powerful tool.

Further studies with a larger number of samples should be conducted to confirm these results and improve their accuracy.

Acknowledgments: NextGenerationEU recovery funds, through the Project BeeLand PRR-C05-i03-I-00008. This work was also supported by Foundation for Science and Technology (FCT, Portugal) for their financial support through national funds FCT/MCTES (PIDDAC) to CERNAS-IPCB, UIDB/00681/2020 (DOI: 10.54499/UIDP/00681/2020).



Technological strategies to increase soluble dietary fibre in cloudy apple juice and effect on oligo- and polysaccharides

**Sónia S. Ferreira¹, Dámaris Maia¹, Rita Bastos², Irina Alves³, Maria João Alegria³,
Elisabete Coelho¹, Manuel A. Coimbra¹**

¹LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Aveiro, Portugal

²Colab4Food – Collaborative Laboratory, INIAV - Vairão, Vila do Conde, Portugal

³SUMOL COMPAL Marcas S.A, Carnaxide, Portugal

* *soniasferreira@ua.pt*

Carbohydrates are key components able to confer sweetness, thickness, and mouth feeling smoothness to fruit juices. Cloudy fruit juices appear as alternative to clarified juices due to their content in total dietary fibre [1]. Nevertheless, the content of dietary fibre with potential prebiotic activity is usually still low [2]. Technological strategies to increment the amount of these compounds are possible based on the modulation of physical treatments of the fruits along juice production.

In this work, cloudy apple juices were produced at a pilot scale plant. The particle size and speed of separation of the apple edible puree were modulated to increase the content of oligosaccharides and soluble dietary fibre.

It was observed that the cloudy juices had a water content of 86%. The total dietary fibre content was 0.4-0.6 g per 200 mL of juice, and its amount is independent of the puree particle size. The soluble dietary fibre comprised 36-49% of dietary fibre, comprising higher percentage when higher particle size purees were processed. The insoluble dietary fibre accounted for 51-63% of dietary fibre, reaching the higher values when lower speed of separation was used, independently of puree particle size. A similar oligosaccharides profile was obtained for all samples. Furthermore, the soluble dietary fibre was mainly composed of pectic polysaccharides rich in galacturonic acid, arabinose and galactose residues.

In conclusion, cloudy apple juice production can benefit of the modulation of the particle size and speed of separation of the edible puree, allowing to produce juices that can have an increment of oligosaccharides and soluble dietary fibre content.

Acknowledgements: The authors acknowledge to the projects of LAQV/REQUIMTE (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020) funded by FCT/MCTES through national funds. S.S.F. would like to acknowledge the research contract funding from VIIAFOOD project. Elisabete Coelho thanks FCT for funding through program DL 57/2016 – Norma transitória (DL 57/2016/CP1482/CT0038).

Funding: This research was funded by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) and the European Union (NextGenerationEU) through the project Agenda VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040).

References:

[1] B. V. Marcotte, M. Verheyde, S. Pomerleau, A. Doyen, C. Couillard, *Nutr.* 14 (2022), 821.

[2] I. Calvete-Torre, C. Sabater, M. J. Antón, F. J. Moreno, S. Riestra, A. Margolles, L. Ruiz, *Food Hydrocoll.* 133 (2022) 107958.

Development of a new Beetroot Snack – Impact of Pulsed Electric Fields (PEF) pretreatment on the Physicochemical and Bioactive Properties

Telma Orvalho¹, Sara Dias¹, Daniela Machado², Inês Soares², Rita Vedor², Ana Gomes², Marco Alves^{1,*}

¹ INOV.LINEA/TAGUSVALLEY – Science and Technology Park, Abrantes, Portugal

² Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

* marco_alves@tagusvalley.pt

Low-caliber vegetables are used to produce juices, flours and dried snacks. The valorization of non- marketable products through the development of new high value-added food products is aligned with current trends and contributes for a more sustainable and circular food sector. PEF is an emerging and promising technology with the potential to reduce freeze-drying time and improve the quality characteristics of processed fruit and vegetables [1,2].

This study evaluated the impact of PEF pretreatment on the physicochemical and bioactive properties of a freeze-dried snack made from low-caliber beetroot. Beetroots, provided by ALITEC (Nazaré, Portugal), were washed, treated with PEF (1.5 kV/cm; 5.4 kJ/kg), sliced, and mixed with sunflower oil and spices. After freeze-drying, with and without PEF pretreatment, analyses were conducted, including water activity (a_w) moisture content, rehydration capacity, hardness, fracturability, total phenolic content (TPC), antioxidant and antidiabetic activities. The samples pretreated with PEF showed a significantly higher a_w (0.27 ± 0.00), moisture content (6.75 ± 0.24 %) and fracturability (29.07 ± 2.636 mm) compared with non-treated samples. Also, PEF caused an increase on TPC and antidiabetic activity, with 5.262 ± 0.128 mg GAE/g of sample and 79.98 ± 1.85 % α -glucosidase inhibition, respectively. The differences in fracturability indicate changes in the cellular structure of the beetroot, giving greater resistance and crunchiness to the final product. The higher antidiabetic activity suggests a beneficial potential of PEF technology in the development of snacks with functional health properties.

This study emphasizes the significance of PEF as a pretreatment for freeze-drying, allowing the valorization of low-value vegetables and the enhancement of the physicochemical and bioactive properties of snacks. PEF treatment has demonstrated potential as a promising approach for developing novel, healthier, and more sustainable food products. Further research is needed to evaluate its impact on freeze-drying cycle duration and cost, ultimately aiming to expand the application of this technology within the food industry.

Acknowledgments: The authors are grateful to ALITEC - Alimentos Tecnológicos SA, providing the raw material for the experiments performed for this work.

Funding: This work was supported financially by the PRR - Plano de Recuperação e Resiliência and by the NextGenerationEU funds at TAGUSVALLEY, through the scope of the Agenda for Business Innovation “Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (VIIAFOOD)” (Project no. 37 AAC n.º 02/C05-i01/2022 with the application C644929456-00000040).

References:

- [1] Bassey, E. J., Cheng, J. H., & Sun, D. W. Novel nonthermal and thermal pretreatments for enhancing drying performance and improving quality of fruits and vegetables. *Trends in Food Science & Technology*, 112 (2021) 137-148.
- [2] Giancaterino, M., Werl, C., & Jaeger, H. Evaluation of the quality and stability of freeze-dried fruits and vegetables pre-treated by pulsed electric fields (PEF). *LWT*, 191 (2024) 115651.

Evaluation of antimicrobial efficacy of current methods to control fire blight (*Erwinia amylovora*)

Daniela Araújo¹, Joana Castro^{1,2}, **João Vindeirinho^{1*}**, Daniel McGuire³, Leonor Cruz³, Carina Almeida^{1,2,4,5}, Hugo Oliveira^{2,6}

¹ INIAV - National Institute for Agrarian and Veterinarian Research, Vairão, Portugal

² CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal

³ INIAV - National Institute for Agrarian and Veterinarian Research, Oeiras, Portugal

⁴ LEPABE— Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁵ ALiCE— Laboratório Associado para a Inovação em Engenharia Química, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁶ LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

* jo.vindeirinho@gmail.com

Erwinia amylovora, a major pathogenic bacterium affecting pome fruit plants, causes fire blight, a devastating disease of pears and apples [1]. Infection typically begins in flowers and leads to blackening of leaves, stems, and immature fruits [2]. *E. amylovora* is widespread in North and Central America and Europe and is spread through insects, rain, wind, infected propagating material, and non-disinfected pruning tools [3].

Several antibiotics have been shown to inhibit the growth of *E. amylovora* *in vitro*, with streptomycin being commonly used in counties like the USA but, still unable to prevent regular outbreaks. Moreover, the use of antibiotics in agriculture is currently restricted in Europe due to concerns about the emergence of antibiotic-resistant strains and their potential impact on human health [5]. Current control methods rely mainly on chemical treatments and few biological control agents. The main goal of this study is to evaluate the antimicrobial susceptibility of 13 *E. amylovora* isolates from symptomatic Portuguese pear orchards to phytosanitary protection products for fire blight control in Portugal.

The screening revealed that copper-based products Bordeaux mixture (copper sulphate) and Cuprital, (copper oxychloride), partially inhibited some isolates. The biological control agent Serenade (*Bacillus amyloliquefaciens* QST 713) was also tested using an antibacterial perpendicular streak assay. The results showed around 15% inhibition on three isolates' growth. As expected, the growth of all isolates tested was inhibited by streptomycin, validating the results obtained in these tests.

These findings underscore the critical nature of current fire blight management, where reliance on prevention rather than effective treatments highlights the idea that innovative, cost-effective, sustainable solutions are needed.

Acknowledgments: This work was funded by national funds through PRR – Recovery and Resilience Plan, under the project PRR-C05-i03-I-000179-LA2.3- BioFago. This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 and UIDB/04050/2020 units, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020 and Associate Laboratory ARNET, LA/P/0069/2020 (<https://doi.org/10.54499/LA/P/0069/2020>). D.A. and J. C. thank FCT for the CEEC Individual (2023.06040.CEECIND and 2022.06886.CEECIND, respectively).

References:

- [1] EPPO Standard Diagnostics (2003). PM 7/20 (3) *Erwinia amylovora*. EPPO Bulletin. 2022;52:198–224.
- [2] S. Myung, J. Y. Lee, M. J. Yun, Y. H. Lee, Y. K. Lee, D. H. Park, C. S. Oh. (2016). Plant Disease, 100(8), 1774-1774.
- [3] D. Shtienberg, S. Manulis-Sasson, M. Zilberstaine, D. Oppenheim, H. Shwartz. (2015). Plant Disease, 99(8), 1048-1058.
- [4] M. Verhaegen, T. Bergot, E. Liebana, G. Stancanelli, F. Streissl, M. P. Mingeot-Leclercq, J. Mahillon, C. Bragard. (2023). Frontiers in Microbiology, 14, 1221478.
- [5] Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território (2009). Produtos autorizados para combate do fogo bacteriano. Artigo 51º do Regulamento (CE) nº 1107/2009.

Oleogels and their different applications: Ham analogues and chocolate spreads

Maria A. Azevedo^{1,*}, Rui Pereira², Rui Rodrigues^{3,4}, Adriana Ferreira⁵, Stephanie Reis¹, Ana Sousa¹

¹ Colab4Food – Laboratório Colaborativo para a Inovação da Indústria Agroalimentar, Vila do Conde, Portugal

² TECMEAT - Centro de Competências do Agroalimentar para o Setor das Carnes, Vila Nova de Famalicão, Portugal.

³ CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

⁴ LABBELS - Associate Laboratory, Guimarães/Braga, Portugal

⁵ Departamento de R&D, Primor Charcutaria Prima – S.A., Vila Nova de Famalicão, Portugal

* alexandra.azevedo@colab4food.com

The World Health Organization (WHO) has raised concerns about excessive fat and salt consumption, prompting the food industry to reformulate products with healthier ingredients. Oleogels are stable and semi-solid structures formed by transforming liquid oils, and they have gained attention as fat replacers. This way, in the present project was tested oleogels in two products: (1) a ham analogue with reduced fat and salt, and (2) a chocolate spread based on oleogel with sunflower or insect oil.

To develop the analogue of ham with fat and salt reduction, a γ -oryzanol+ β -sitosterol oleogel was used to replace fat and a seaweed mixture to reduce the amount of salt. The oleogel was developed with 90% sunflower oil and 10 % structuring agent. In the standard formula of ham analogue, the sunflower oil was replaced by 50 % water and 50 % oleogel. The seaweed mixture replaced 80 % of salt. The use of oleogel allowed a fat reduction of 40 % being the hardness of ham analogue around 150 ($\pm 7,19$) N. Relatively to the firmness, it was very similar ($p < 0.05$) to the standard formula.

The chocolate spread based on oleogel was developed with oleogel of sunflower (SfO) or *Tenebrio Molitor* (IO) oil plus white beeswax (21 %), chocolate (42 %), vegetal fiber (36 %), and salt (1 %). No differences ($p < 0.05$) were detected in the firmness of SfO and IO spreads, however, SfO and IO are more spreadable than commercial control (CC). All samples showed a similar structure exhibiting the SfO and IO spreads a stronger gel strength when compared to the CC. It is possible to develop chocolate spreads based on oleogels with less fats and sugar while maintaining good characteristics and consumer acceptance.

These findings suggest that oleogels can be successfully incorporated into food formulations to create healthier alternatives without compromising sensory qualities.

Acknowledgements: VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação Comercial para o AgroAlimentar (C644929456-00000040), financiado pelo PRR - Plano de Recuperação e Resiliência e pelos Fundos Europeus NextGeneration EU.

Nutritional and antioxidant properties of clean-label yogurt added fermented fruit preparations

Cristiana Santos^{1,*}, Rita Barracosa², Daniela Correia², Luís Paulico³, Cristina Rodrigues³, Anabela Raymundo¹, Catarina Prista¹, Juliana Botelho Moreira¹

¹LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisbon, Portugal

²MC Shared Services S.A., Rua João Mendonça, 529, 4464-501 Senhora da Hora, Matosinhos, Portugal.

³FRULACT • Headquarters & Frutech RDI Centre, Rua do Outeiro, 589 • 4475-150 Gemunde—Maia, Portugal

* *cristiana.filipe.santos@gmail.com*

The demand for clean-label food products has increased since consumers look for healthier, more natural, and sustainable foods. Yogurts are widely consumed, however, those with fruit pulps (in particular) may contain chemical additives, including preservatives, which concern consumers due to their potential negative effects on human health [1]. LAB fermentation is a potential approach in this context because LAB produce various antimicrobial metabolites against spoilage microorganisms [2], like bacteriocins, organic acids, and others, that also present improved bioactivity.

This work aimed to evaluate the effect of adding fermented fruit preparations to yogurts on their preservation, nutritional composition, and antioxidant properties. The fruit preparations were fermented for 4 days with two microbial consortiums: Bifidobacterium (BIF) and Kefir (KF). After that, the fermented fruit preparations were autoclaved and added to Greek (GY) and drinkable (DY) base-yogurts, obtaining four clean-label products (BIF-GY, KL-GY, BIF-DY, and KL-DY). The clean-label yogurts were carried out regarding nutritional composition and antioxidant activity, through DPPH, FRAP method and total phenolic content, and shelf-life by drop tests.

Adding fermented fruit preparation to yogurts improved their nutritional value and bioactivity. BIF-DY and KL-DY demonstrated higher total protein values (61%) than the control-DY (56%). The lipid content of BIF-DY and KL-DY decreased from the control-DY by 200% and 100%, respectively, but increased for GY. Additionally, all yogurts had significantly enhanced mineral content (Na, K, Ca, P, and Zn). The FRAP results increased by 37.2% and 38.7% in BIF-GY and KL-GY, respectively. Furthermore, the total phenolic content improved in clean-label yogurts, with higher values observed in BIF-GY and KL-GY (24.39 ± 0.69 and 22.40 ± 0.57 mg GAE/100 g, respectively). Also, the addition of BIF and KL fermented fruit pulp, improved the preservation ability of the yogurts against food contaminants. The use of fermented pulps appears to be a valid strategy for clean-labelling.

Acknowledgements: We thank Linking Landscape, Environment, Agriculture and Food - LEAF (UIDB/04129 / 2020), MC SONAE, and Frulact company. This work was supported by Agendas Mobilizadoras para a Inovação Empresarial -PRR I Aviso No 02/C05-i01/2022 project VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040), WP1 – A1.8. Development of clean label food products.

References:

- [1] M-Y. Chang, C-C. Huang, Y-C. Du, H-S. Chen, *nutrients* 14 (2022) 3523.
- [2] Zapaśnik, B. Sokołowska, M. Bryła, *Foods*, 11 (2022) 1283.

Formulation and characterization of two clean-label, low-fat butter alternatives

Leila Oliveira¹, Rita Barracosa², Daniela Correia², Cristiana Santos^{1,*}, Catarina Prista¹, Anabela Raymundo¹, Juliana Botelho Moreira¹

¹LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisbon, Portugal

²MC Shared Services S.A., Rua João Mendonça, 529, 4464-501 Senhora da Hora, Matosinhos, Portugal

* *cristiana.filipe.santos@gmail.com*

The demand for healthier foods without chemical additives has led to the popularity of clean-label foods. The main objective of this work was to develop and characterize clean-label low-fat butters produced using green banana flour (GBF) and inulin (I) to improve the nutritional and technological characteristics of food products. The addition of whey protein (WP) and gelatin (G) was also evaluated. Twelve formulations were prepared, varying the content of GBF or I (1 and 1.5%), WP (3, 4, and 5%), and G (0 and 1%), of which eight were selected for further physicochemical, rheological, and sensory characterization, comparing with the commercial target product (MMC).

The addition of inulin reduced moisture without changing the fat content of low-fat butters. All clean-label butters presented higher fiber content. Notably, a significantly increased in the fibre content was observed when Inulin was used. (500% higher than with GBF). All clean-label butters showed high protein content (2.9–5.5 g/100 g), pH higher than MMC, and $\Delta E > 5$, which indicates a noticeable color change that still needs to be corrected in the future. The addition of gelatin increased the G' modulus in formulations with 1% (GBF or inulin) and 3% WP. In the sensory analysis, the butters showed high acceptance, with emphasis on the formulations with GBF, which obtained higher purchase intention. Two formulations, one with GBF and WP (F6) and the other with I+WP+G (F12), were evaluated for stability (0 and 21 days), with an increase in the pH value, in lean butter with inulin, and a reduction in moisture content, in butter with GBF. Thus, this study proved that both GBF and inulin have the potential to develop clean-label lean butters, contributing sustainably to society, industry, and the environment.

Acknowledgements: We thank Linking Landscape, Environment, Agriculture and Food - LEAF (UIDB/04129 / 2020), MC SONAE, and Frulact company. This work was supported by Agendas Mobilizadoras para a Inovação Empresarial -PRR I Aviso No 02/C05-i01/2022 project VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040), WP1 – A1.8. Development of clean label food products.

Factorial Design Study of Pulsed Electric Fields Parameters to Improve the Rehydration Ratio of Freeze-Dried Banana Slices

Diogo Gonçalves, Viviana Monteiro, Marco Alves

NOV.LINEA/TAGUSVALLEY – Science and Technology Park, Abrantes, Portugal
marco_alves@tagusvalley.pt

Rehydration ratio is an important factor in the quality and economic viability of dried food products, as it directly impacts logistics costs and consumer satisfaction. Improving rehydration not only reduces transportation and storage expenses by minimizing weight and volume but also enhances product value for consumers. The aim of this study is to investigate the impact of pulsed electric fields (PEF) pre-treatment on the rehydration properties of freeze-dried banana slices. A fractional factorial design (2^{4-1}) was applied to evaluate four PEF variables: temperature (20-40 °C), field intensity (1.5-7.5 kV/cm), number of pulses (50-200), and frequency (10-100 Hz), with rehydration ratio as the primary response variable. A multiple linear regression (MLR) analysis was used to fit a mathematical model to the experimental data, and the model's validity was tested using analysis of variance (ANOVA). The rehydration ratios were determined by immersing 3 g of dried banana slices in 400 mL of distilled water at 20 °C for 60 minutes. The results demonstrated that PEF pre-treatment increased the rehydration ratio by up to 64% compared to the control sample (freeze-dried without PEF pre-treatment), with optimal conditions identified at 20 °C, 1.5 kV/cm and 200 pulses. Temperature was the most influential factor, while frequency exhibited negligible impact within tested ranges. A difference of 41% in rehydration ratio was observed between the least and most effective PEF conditions. This study highlights the utility of factorial design as a cost-effective method for optimizing PEF parameters and supports the potential of PEF as an effective pre-treatment to enhance rehydration efficiency, offering economic and practical benefits for both consumers and producers in the dried food industry. Future research should investigate the optimizing of PEF parameters in a broader range of food matrices and drying techniques to maximize PEF industrial acceptability.

Funding: This work was supported financially by the PRR—Plano de Recuperação e Resiliência and by the NextGenerationEU funds at TAGUSVALLEY, through the scope of the Agenda for Business Innovation “Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (VIIAFOOD)” (Project no. 37 AAC n.º 02/C05-i01/2022 with the application C644929456-00000040).

Moderate pressure pasteurization combined with oxygen scavenger: an innovative approach to improve physical properties of salmon

Alireza Mousakhani Ganjeh^{1,2}, Carlos A. Pinto², Susana Casal¹, Jorge A. Saraiva^{1,*}

¹ LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

² LAQV-REQUIMTE, Department of Chemistry, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal

* *Jorgesaraiva@ua.pt*

As consumer demand for high-quality, minimally processed seafood continues to rise, it is essential to preserve the sensory and nutritional attributes of seafood [1]. High-pressure processing (HPP) often negatively affects these qualities due to extensive protein denaturation and oxidative damage [1,2]. In contrast, moderate-pressure pasteurization (MPP), applied at pressures ranging from 100 to 250 MPa over extended periods, provides a gentler alternative, although it may still lead to some undesirable color changes. Integrating an oxygen scavenger (OS) into the packaging environment can reduce oxidative processes that lead to lipid oxidation and pigment degradation [3].

Therefore, this study explores an innovative preservation strategy that combines MPP with OS to improve the overall quality of salmon.

In this study, salmon samples underwent several treatments, including MPP (100 MPa for 3 days) with different packaging conditions (normal, vacuum, and OS), and HPP (600 MPa for 5 min). Microbial analyses confirmed that all pressure-based treatments significantly reduced total aerobic mesophiles (>5 log) and Enterobacteriaceae, effectively ensuring food safety. Colorimetric assessments indicated that HPP-treated samples exhibited marked increases in lightness and whiteness, attributed to severe protein denaturation and pigment degradation. In contrast, MPP/OS samples showed notably lower ΔL and ΔE values, reflecting minimal color deviation from fresh salmon.

Further evaluations of drip loss, cooking loss, and water-holding capacity revealed that the combination of MPP with OS effectively minimized moisture loss and better preserved structural integrity compared to other methods. Texture profile analyses indicated that while HPP significantly increased hardness and chewiness, the incorporation of OS within MPP helped maintain desirable textural attributes.

In summary, the synergistic use of MPP alongside OS demonstrates a capability to retain the natural color, texture, and moisture of salmon, all while ensuring microbial safety. These findings position the MPP/OS method as a promising, sustainable alternative for the seafood industry.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Thanks are also due to the FCT/MCTES for the PhD fellowships of Alireza Mousakhani Ganjeh (2022.12558.BD) and Carlos A. Pinto (SFRH/BD/137036/2018 and COVID/BD/153220/2023).

References:

- [1] Mousakhani Ganjeh, C.A. Pinto, S. Casal, J.A. Saraiva, Food Bioscience, 61 (2024) 104537.
- [2] Mousakhani Ganjeh, C.A. Pinto, S. Casal, J.A. Saraiva, Food Bioscience, 59 (2024) 103963.
- [3] Dey, S. Neogi, Trends in Food Science & Technology, 90 (2019) 26-34.

Black soldier fly larvae oil: A game-changer in enhancing fillet quality of high-value mediterranean fish

Ana Basto^{1,2*}, Daniel Acebes^{1,2}, Andreia Filipa-Silva¹, Paula Canada¹, Sara Magalhães³, Daniel Murta⁴, Luísa M.P. Valente^{1,2}

¹CIIMAR/CIMAR LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal

²ICBAS, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

³SORGAL, Sociedade de Óleos e Rações S.A., S. João de Ovar, Portugal

⁴EntoGreen®, Ingredient Odyssey S.A., Santarém, Portugal

* ana.basto@ciimar.up.pt

Recent changes in aquafeed formulations have led to a reduction in omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) and an increase in omega-6, resulting in a significant imbalance in their ratio in fish flesh. This imbalance can reach ratios far from the recommended range of 1:4-5 for a healthy human diet [1]. Moreover, fish is highly perishable, highlighting the need for innovative and effective strategies to maintain its nutritional quality and extend shelf life. Black soldier fly larvae oil (BSFLO) is rich in lauric acid - a medium-chain fatty acid preferentially utilized for β -oxidation - and contains tocopherols with strong antioxidant properties [2]. This study hypothesized that BSFLO could act as a sparing promoter and natural antioxidant of omega-3 LC-PUFA when included in diets for European seabass and gilthead seabream, two key species in Mediterranean aquaculture.

Four isoenergetic diets were tested in triplicate in two parallel feeding trials [European seabass (initial body weight (IBW) = 11 ± 0.2 g) and gilthead seabream (IBW = 18 ± 0.3 g)]: a commercial-like control diet and three experimental diets containing 1.7%, 3.4%, and 5.2% BSFLO, at the expense of poultry oil. After 10 weeks of feeding, 15 fish per treatment were euthanized for white dorsal muscle sampling and further analysis of total lipids, fatty acid profile, lipid peroxidation (LPO), and oxygen radical absorbance capacity (ORAC) analysis [3].

The results demonstrated that dietary inclusion of BSFLO did not affect the total muscle lipid content in either fish species. However, the inclusion of 5.2% BSFLO significantly increased lauric acid levels and the omega-3:omega-6 ratio in the muscle of both species, reduced lipid peroxidation, and enhanced antioxidant capacity, supporting the study's hypothesis.

Acknowledgments: This research was funded by the InsectERA Mobilising Agenda, framed in the Portuguese Recovery and Resilience Plan (PRR), with reference: C644917393-00000032, and supported by the strategic funding UIDB/04423/2020 and UIDP/04423/2020, and LA/P/0101/2020 (<https://doi.org/10.54499/LA/P/0101/2020>) through national funds provided by FCT – Fundação para a Ciência e a Tecnologia.

References:

- [1] I. Carr, B. Glencross, E. Santigosa, *Frontiers in Animal Science*, 4 (2023), 1147081.
- [2] A. Benzertiha, B. Kierończyk, M. Rawski, Z. Mikołajczak, A. Urbański, L. Nogowski, D. Józefiak, *Annals of Animal Science*, 20:4 (2020), 1217.
- [3] A. Filipa-Silva, A. Marques, M. A., Salgado, H. Abreu, J. Dias, L. M. P. Valente, *Frontiers in Sustainable Food Systems*, 7 (2023), 1224370.

This work was developed in collaboration with industry.

Advancing algae-based foods: Bridging academia and industry for culinary innovation

José Matheus^{*1}, Maria João Alegria², Maria Cristiana Nunes¹, Anabela Raymundo¹

¹ LEAF - Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal

² SUMOL+COMPAL, Portugal

* jmatheussilva@isa.ulisboa.pt

This research is part of the Sea2Fork initiative, a dynamic collaboration between academia and industry, dedicated to developing pre-prepared and ready-to-eat meals by combining algae with vegetable-based raw materials. This partnership drives both scientific advancements and innovative industrial approaches in food development. It represents a significant breakthrough for SUMOL+COMPAL, delivering nutritionally balanced products with distinctive sensory qualities while fostering a shift in consumer culinary preferences. Integrating seaweeds and microalgae into food formulations is a very important step toward enhancing sustainability, advancing the blue bioeconomy, and leveraging their numerous health benefits. This study explores the development of algae-enriched food products, including tomato pulps, hummus with pulses, soups, and other ready-to-eat meals, by developing both nutritious and appealing alternatives while supporting the transition to more sustainable food systems, from laboratory formulations to their feasibility for industrial-scale production. Key attributes such as nutritional content, antioxidant capacity, texture, rheological properties, color, and sensory appeal were evaluated and compared to traditional, algae-free alternatives. The findings highlight the functional and gastronomic potential of these algae-based solutions and, for most products, the incorporation of these ingredients leads to an increase in protein content, minerals, and antioxidant activity directly attributed to algae incorporation. However, their inclusion also requires formulation adjustments to optimize food structure and sensory characteristics, as consumer acceptance ultimately depends on taste [1]. By promoting these challenges into a market integration, this approach aims to position algae as a valuable ingredient in the food industry [2].

Funding: This work was financially supported by “Pacto da Bioeconomia Azul” (Project No. C644915664-00000026) within the WP5 Algae Vertical, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation.

Acknowledgments: Portuguese Foundation for Science and Technology (FCT), UIDB/AGR/04129/2020 LEAF Research Unit.

References:

- [1] J. Matheus, M. J. Alegria, M. C. Nunes, A. Raymundo, Algae-Boosted Chickpea Hummus: Improving Nutrition and Texture with Seaweeds and Microalgae, *Foods*, 13 (2024) 2178.
- [2] M. Mendes *et al.*, Algae as Food in Europe: An Overview of Species Diversity and Their Application, *Foods*, 11 (2022) 1871.



Encapsulated aromas for enhancing canned tuna flavour and reducing salt

Beatriz Fernandes^{1,2}, Gabriel Mota Ribeiro³, Daniela Araújo⁴, Joana Castro^{4,5}, Luis F. Baião^{6,7}, Helga Augusto⁸, Ana Cação⁸, Sara Sousa⁹, Valentina F. Domingues⁹, Cristina Delerue-Matos⁹, Juliana Pais Costa¹⁰, Irina Zabelo¹¹, Helena Oliveira^{10,11}, Leonor Nunes¹⁰, Carmo Serrano^{2,12*}

¹ INIAV, National Institute for Agrarian and Veterinary Research, Oeiras, Portugal

² CERENA, DEQ, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal

³ FMSI, Lda, Rio de Mouro, Portugal

⁴ INIAV, National Institute for Agrarian and Veterinary Research, Vairão, Portugal

⁵ Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal

⁶ SenseTest Lda, Vila Nova de Gaia, Portugal

⁷ GreenUPorto/INOVA4Agro & DGAOT, Faculty of Sciences of the University of Porto, Vairão, Portugal

⁸ Cofisa, S.A, Figueira da Foz, Portugal

⁹ REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Porto, Portugal

¹⁰ Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, University of Porto, Matosinhos, Portugal

¹¹ Division of Aquaculture, Upgrading, and Bioprospecting, Portuguese Institute for the Sea and Atmosphere, Lisbon, Portugal

¹² LEAF- Linking Landscape, Environment, Agriculture and Food Research Unit, Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

**carmo.serrano@iniav.pt*

Tuna is rich in polyunsaturated fatty acids, which help manage conditions such as cardiovascular, inflammatory diseases and diabetes [1]. Unfortunately, canned products often contain high levels of sodium, which may increase blood pressure and heart diseases. As a result, the food industry is exploring salt alternatives without compromising taste. One promising approach is the use of plant extracts to enhance flavour, minimizing the need for excess salt. Essential oils (EOs) and oleoresins (ORs) are natural extracts rich in bioactive compounds that provide preservative effects, in addition to boosting flavour [2]. However, these sensitive compounds deteriorate during canning processes and can oxidize over time, so encapsulation is an excellent solution to protect them, guaranteeing their long-term stability and providing controlled release of flavours and bioactive compounds.

In this work, a blend of lemon EO and pepper OR was formulated and subsequently encapsulated for use in a salt-reduced canned tuna product. The tuna containing microencapsulated extracts (MEs) was compared with tuna products prepared with synthetic flavourings (control). The physicochemical properties of the MEs were analysed, including their toxicity, particle size distribution by dynamic light scattering, while mineral content of the canned tuna was tested and compared to the control group. A consumer acceptance study was also conducted to evaluate the potential of MEs as flavour enhancers and their effectiveness in reducing salt content.

The analysis of the MEs confirmed their bioactive composition, powder stability, and their non-toxic nature. Chloride and sodium levels in canned tuna with MEs were lower than in the control group, while potassium levels were higher. Consumer acceptance testing revealed no significant differences between the MEs tuna cans and the control canned tuna. Overall, these results suggest that the use of MEs from plants can reduce sodium content by 28% without compromising consumer acceptability.

Acknowledgments: The authors would like to acknowledge FCT for supporting the project “Pacto da Bioeconomia Azul” (PRR-C644915664-00000026). D. Araújo and J. Castro would like to thank FCT for the CEEC Individual (2023.06040.CEECIND, 2022.06886.CEECIND, respectively).

References:

- [1] Maqbool, B. Strandvik, V. A. Stallings, The skinny on tuna fat: health implications. *Public Health Nutrition*, 14 (2011).
- [2] Serrano, M. Sapata, D. Castelo-Branco, A. Tasso, A. Marques, C. Viegas, D. Figueira, N. Komora, The influence of salt reduction with encapsulated oleoresins on the quality of mayonnaise, mustard, and ketchup. *Frontiers in Nutrition*, 11 (2024).

C-Phycocyanin as a natural alternative to the synthetic blue food dye E133 in beverages

Mariana Pereira¹, Marisa Cristo¹, Catarina Teixeira-Guedes^{1,2}, Rui Rodrigues^{1,2},
Cristina M. R. Rocha^{1,2}, Daniela Correia³, José A. Teixeira^{1,2}, Fernanda Lüdtke^{1,2},
Daniela Martins^{1,2,*}

¹CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

²LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

³MC Shared Services S.A., Lugar do Espido, Via Norte, 4470-177 Maia, Portugal

* dsr.martins@ceb.uminho.pt

E133 is a synthetic blue dye derived from petroleum, commonly used in processed foods such as candies, desserts and soft drinks. Replacing it with a natural alternative would be a step towards eliminating synthetic additives and preservatives in food products. *Arthrospira platensis*, also known as Spirulina, contains C-phycocyanin, a protein-pigment complex that acts as a natural blue dye with antioxidant properties, making it a promising substitute for synthetic dyes. However, the major drawback of C-phycocyanin is the low colour stability, especially in beverages, requiring strategies to improve storage stability [1, 2].

In this work, C-phycocyanin was extracted through clean processes, using water as solvent through freeze-thawing, centrifugation and ultrafiltration. The extract was incorporated into a colorless isotonic drink, with the concentration adjusted to achieve a colour comparable to a commercial drink containing the synthetic dye. Stability tests were performed at different pH levels, with greater stability being observed at pH=4. The drinks were subjected to different temperatures and light conditions, and it was confirmed that the pigment is affected by light exposure and high temperatures, particularly during pasteurization.

To address this, two strategies were implemented to extend the pigment stability: microencapsulation in a polysaccharide matrix and the entrapment in nanoemulsions. Encapsulation in a polysaccharide matrix composed of maltodextrin and xanthan gum (75:25) or xanthan gum alone significantly improved stability to pasteurization and storage, preserving around 60 % of the initial color after 30 days (40 % more than the free pigment). Entrapment of C-phycocyanin in oil-in-water nanoemulsions resulted in bright blue-colored samples with droplet sizes < 250 nm. Preliminary tests with storage at 4 °C showed color and size stability for a minimum of 15 days.

These methodologies proved promising for improving the stability of C-phycocyanin in solution and may contribute to its use as a replacement for artificial dyes.

Acknowledgments: This work was financially supported by Agenda VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (nº C644929456-00000040), funded by the NextGeneration EU European Fund and the Portuguese Recovery and Resilience Plan (PRR). CEB and LABBELS are also supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and LA/P/0029/2020, respectively.

References:

- [1] B. Fernández-Rojas, J. Hernández-Juárez, J. Pedraza-Chaverri, Journal of Functional Foods, 11 (2014).
- [2] D. P. Jaeschke, I. R. Teixeira, L. D. F. Marczak, G. D. Mercali, Food Research International, 143 (2021).

Exploring the use of oleogels as alternatives to palm oil in chocolate spread formulations

Isabel S. Franco¹, Rui M. Rodrigues^{2,3}, Jorge M. Vieira^{2,3}, Manuel Rendueles¹,
José A. Teixeira^{2,3}, Fernanda L. Lüdtkke^{2,3}, *

¹Department of Chemical and Environmental Engineering, University of Oviedo, C/Julián Clavería 8, 33006 Oviedo, Spain

²CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

³LABELS – Associate Laboratory, Braga/Guimarães, Portugal

* fludtke@ceb.uminho.pt

Palm oil and its stearins are widely used in chocolate spreads due to their favorable physical properties. However, concerns over their high saturated fatty acid content and sustainability issues have led to the emergence of oleogels as an alternative [1]. Oleogels are formed by trapping vegetable oil within a crystalline network created by a structuring agent, resulting in a semi-solid lipid with low saturated fatty acid and zero trans-fat content, making them suitable for incorporation into food formulations [1,2].

In this context, the aim of this study was to evaluate the potential of oleogels as a replacement for palm oil in chocolate spread formulations. First, the study assessed the effect of different structuring agents (rice wax and glycerol monostearate) at varying concentrations (3%, 5%, and 7%) on the stability, thermal, rheological, and textural properties of the oleogels. Second, the selected oleogels were incorporated into chocolate spread formulations and stored at 20°C to evaluate their stability, rheological behavior, and texture over storage time.

The results indicated that the type of structuring agent and its structural characteristics had a direct impact on the rheological and textural properties of the produced oleogels. Specifically, when compared at the same concentration (e.g., 7%), the glycerol monostearate-based oleogel exhibited behavior more similar to that of palm oil, whereas the rheological and textural analyses of the rice wax-based oleogel revealed contrasting behavior. Characterization data showed that a concentration of 7% (w/w) resulted in more stable oleogels for both rice wax and glycerol monostearate. Furthermore, the physicochemical properties of the structuring agents influenced the firmness and rheological behavior of oleogel-based chocolate spreads.

Therefore, the substitution of palm oil with oleogels enabled the development of stable chocolate spreads with desirable characteristics, offering a nutritionally healthier alternative by replacing saturated fats, while also providing a more environmentally sustainable option.

Acknowledgments: This work was financially supported by Agenda VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040), funded by the NextGeneration EU European Fund and the Portuguese Recovery and Resilience Plan (PRR). CEB and LABELS are also supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and LA/P/0029/2020, respectively.

References:

- [1] R.C. Silva, M.J. Ferdaus, A. Foguel, T.L.T. Silva, *Gels*, 9 (2023).
- [2] E. Flöter, T. Wettlaufer, V. Conty, M. Scharfe, *Molecules*, 26 (2021).

Sodium reduction in canned tuna: effective strategies for a healthier product

H. Oliveira^{1,2,*,#}, I. Zabelo^{1,2,#}, A.J. Pais-Costa², C. Camacho^{1,2}, C. Pires^{1,2}, H. Lourenço¹, M.J. Fraqueza³, M.H. Fernandes³, M.J. Fernandes³, A. Cação⁴, H.C. Augusto⁴, L.F. Baião^{5,6}, M.P. Duarte⁷, A. Gonçalves^{1,2}, A. Marques^{1,2}, M.L. Nunes¹

¹IPMA, IP - Portuguese Institute for the Sea and Atmosphere, Division of Aquaculture, Upgrading and Bioprospection, Algés, Portugal

²CIIMAR/CIMAR-LA - Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal

³CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, AL4AnimalS - Associate Laboratory for Animal and Science, University of Lisbon, Lisboa, Portugal

⁴COFISA - Conservas de Peixe da Figueira, S.A., Figueira da Foz, Portugal

⁵SenseTest Lda. - Sociedade de Estudos de Análise Sensorial a Produtos Alimentares, Lda., Vila Nova de Gaia, Portugal

⁶GreenUPorto/INOV4Agro & DGAOT, Faculty of Sciences of the University of Porto, Vairão, Portugal

⁷MEtRICs - The Mechanical Engineering and Resource Sustainability Centre, Chemistry Department, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal

*helena.oliveira@ipma.pt

#Equal contribution

Sodium chloride (NaCl) is widely used in several stages of canned fish production. However, excessive sodium (Na) intake is a growing public health problem worldwide that must be counteracted [1]. This fact has led the industry to look for ingredients to partially or totally replace NaCl, while maintaining the product quality and safety. The use of food-grade potassium chloride (KCl) and flavour enhancers has been suggested as an effective strategy to reduce Na content in processed foods [2,3]. In this context, the aim of this work was to: i) develop canned tuna (*Katsuwonus pelamis*) in oil with reduced Na content by replacing NaCl (50% and 100%) with KCl and a flavour enhancer (commercial protein hydrolysate (HP)); ii) evaluate the effect of these substitutions on stability, sterility, physicochemical and sensorial properties of the prototypes. To this end, ¼-club cans with five formulations (including one control, 100% NaCl) were prepared in an industrial environment (Cofisa, S.A.) (~200 cans/formulation). After cooling, cans were kept for one month at room temperature (business as usual) and analysed.

The results showed that all prototypes were stable and sterile. In general, the proximate chemical composition, physical parameters (instrumental colour and texture), sensory properties (assessed by a trained panel; e.g., salty taste, bitter taste, firmness, juiciness) and overall acceptability (evaluated by a consumer panel) of the prototypes were not affected by the formulations tested. Replacing 50% and 100% of NaCl with KCl or HP led to similar reductions in Na (around 30% and 48%, respectively), making it possible to state that the developed canned prototypes can be easily marketed as “reduced in Na content”, in accordance with the Regulation (EC) No. 1924/2006 [4]. Additionally, the formulations with KCl led to increases in K by 47% and 83%, respectively, which could be a major contribution for cardiovascular health [5].

Acknowledgments/Funding: This work has been developed within the scope of “BLUE BIOECONOMY INNOVATION PACT” (Project N.º. C644915664-00000026) financed by NextGenerationEU, under the incentive line “Agendas for Business Innovation” of the Recovery and Resilience Plan (PRR). This work was also supported by Portuguese Foundation for Science and Technology (projects: UIDB/04077/2020, UIDB/04423/2020 and UIDP/04423/2020).

References:

- [1] World Health Organization, Sodium reduction (2023), URL: <https://www.who.int/news-room/fact-sheets/detail/salt-reduction> (Accessed January 29, 2025).
- [2] Estévez, C. Camacho, T. Correia, et al., Food Chem. Toxicol., 153 (2021) 112262.
- [3] K. Cepanec, S. Vugrinec, T. Cvetković, J. Ranilović, Compr. Rev. Food Sci. Food Saf., 16 (2017) 881–894.
- [4] Regulation (EC) No 1924/2006. Official journal of the European Union, 404 (2006) 9–25.
- [5] European Food Safety Authority, EFSA Supporting Publications, 14 (2017) 12.

Development of protein bars with insect flour (*Tenebrio molitor*): Microbiological, nutritional and sensory evaluation

**Vinhas. A.^{*1}, Guimarães. A.¹, Mota A.¹, Teixeira M.¹, Barbosa B.^{2,3}, Rocha C.^{2,3},
Lima R.²**

¹Associação Colab4Food – Laboratório Colaborativo Para a Inovação da Indústria Agroalimentar, Porto, Vila do conde

² Sense Test, Lda, Rua Zeferino Costa, 341, 4400-345 Vila Nova de Gaia

³ GreenUPorto - Sustainable Agrifood Production Research Centre / Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Campus de Vairão, Rua da Agrária 747, 4485-646 Vila do Conde

**ana.vinhas@colab4food.com*

The research for sustainable protein sources has gained prominence in recent decades in response to global concerns about food security and environmental sustainability. In this context, insects have emerged as a promising option due to their high protein content, essential amino acid profile, and low ecological footprint. This study aimed to develop protein bars incorporating *Tenebrio molitor* larva flour in different concentrations and to evaluate their sensory, microbiological, and nutritional characteristics. These bars were formulated containing 0%, 5%, 10%, and 15% insect flour, to assess the impact of adding this ingredient on the properties of the products, as well as on consumer liking and perception.

The bars underwent microbiological analysis to ensure food safety. The analyses followed the regulated microbiological standards for ready-to-eat foods. The results indicated that all samples were within acceptable limits for human consumption, confirming that the production process effectively ensured the microbiological safety of the bars.

In terms of nutritional and sensory profile, the bars with 10% insect flour were, overall, the most promising. *Tenebrio molitor* flour is rich in high-quality proteins, as well as unsaturated fatty acids and micronutrients such as iron and zinc. The inclusion of this ingredient increased the nutritional value of the bars without substantially changing the energy content. This suggests that insect flour can be an efficient alternative to improve the nutritional profile of food products, meeting the needs of consumers seeking functional foods and alternative protein sources.

This study demonstrated that protein bars with *Tenebrio molitor* flour is feasible and that it is essential to find a balance between nutritional value and food safety, without impacting consumer liking, despite some sensory differences perceived in the different formulations. The results of this study indicate that insect-based products have the potential to become an alternative to human nutrition.

Acknowledgments: This work was financially supported by “InsectERA” (Project No. C644917393-00000032) within the WP InFood, funded by Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line “Agendas for Business Innovation” through the funding scheme C5 - Capitalization and Business Innovation. Author Bruna Barbosa acknowledges Non-Academic Doctoral grant No. 2023.03467.BDANA, funded by the Foundation for Science and Technology (FCT) and supported by Sense Test, Lda. Authors, Bruna Barbosa and C. Rocha acknowledge additional support from FCT through UID 05748: GreenUPorto - Centro de Investigação em Produção Agroalimentar Sustentável.

Transforming orange and banana peels fermented with *Yarrowia lipolytica* into nutritious snack bars

**Marlene Lopes^{1,2*}, Liliana P. Araújo¹, Andreia S. Fernandes¹, Joana Solinho³,
Isabel Belo^{1,2}, Rita Pinheiro³**

¹ CEB—Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057
Braga, Portugal

² LABBELS—Associate Laboratory, Braga/Guimarães, Portugal

³ Center for Research and Development in Agrifood Systems and Sustainability, Polytechnic
Institute of Viana do Castelo, Avenida Atlântico, 4900-348 Viana do Castelo, Portugal

* marlenelopes@ceb.uminho.pt

Consumers are more aware of the nutritional value, safety, and sustainability of food products, seeking options that deliver essential nutrients, are free from harmful additives, and are produced through eco- friendly practices. This shift toward healthier eating habits has fuelled the rapid growth of the plant-based food industry. Microbial fermentation has emerged as a promising strategy to enhance the nutritional profile of food products, such as boosting protein content and reducing sugar levels. It offers a sustainable and safe approach to developing products that align with health-conscious consumers' growing demand for balanced and responsible food choices. In this sense, this study assessed the potential of solid-state fermentation (SSF) using *Yarrowia lipolytica*, a GRAS-status yeast approved as a novel food by EFSA in 2019, to enhance the nutritional value of banana (BP) and orange (OP) peels. Both peels are a good source of minerals, fibres, and carbohydrates, but are poor in protein (8% BP and 5% OP). Following SSF optimization, including studies on medium supplementation, mixture, and peel pre-treatments, protein content increased to 10.2% in BP and 9.4% in OP. Additionally, significant reductions in carbohydrates and soluble sugars were achieved. Fermented BP and OP (fruit peels+yeast) were used as the main raw material to develop two snack bars formulated with: (1) fermented OP and BP, oat flakes, chocolate, peanut butter, toasted sunflower seeds, orange juice, agave syrup, and whey; (2) fermented BP, banana pulp, chocolate, and sliced almond. A taste test involving 190 consumers revealed strong acceptance of the new snacks, particularly formulation (2), and highlighted a high willingness to purchase this innovative product, which differentiates itself from current market options. Fermented fruit peels with *Yarrowia lipolytica* marks a step forward in food biotechnology, enabling the development of innovative, nutrient-rich products while supporting environmental sustainability through the valorization of underutilized food-grade by-products.

Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and project Micro4Food - Upgrading fruit wastes for microbial protein production (2022.01705.PTDC).

Sodium selenate vs sodium selenite: Which is the best choice for selenium biofortification of Swiss chard microgreens?

Alexis Pereira^{1,2}, Maria Inês Dias¹, M. Beatriz P.P. Oliveira², José Pinela^{1,3,*}

¹ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

² REQUIMTE/LAQV, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

³ National Institute for Agricultural and Veterinary Research (INIAV), I.P., Rua dos Lágidos, Lugar da Madalena, 4485-655 Vairão, Vila do Conde, Portugal

* jose.pinela@iniav.pt

Selenium (Se) is an essential micronutrient with critical biological functions in humans [1]. However, its deficiency affects millions of people worldwide and is associated with serious health issues [2-3]. Climate change may further exacerbate this problem by depleting Se levels in agricultural soils, thereby reducing its availability in food. As a result, addressing food insecurity and malnutrition is a key priority in the UN's 2030 Agenda for Sustainable Development. This study aimed to identify the inorganic forms of Se most efficiently absorbed by Swiss chard (*Beta vulgaris* subsp. *cicla*) microgreens and to assess their impact on the composition of minerals, phenolic compounds, and betalains.

Microgreens were grown in trays filled with vermiculite within a walk-in growth chamber equipped with LED lamps. The plants were irrigated with an NPK 3-1-6 nutrient solution supplemented with 10, 20, 40 μM Se, provided as sodium selenate, sodium selenite, or a combination of both at 20 μM Se. A photoperiod of 14/10 h (light/dark) was applied, with temperature and relative humidity controlled between 20-25 °C and 60%, respectively. Fourteen days after sowing, microgreens were hand-harvested, and the phytochemical analyses were performed using atomic absorption spectroscopy (for minerals) and high-performance liquid chromatography (for the remaining constituents).

Up to eight phenolic compounds, including phenolic acids and flavonoids, and seven betalains were identified, with their concentrations varying among treatments. The biofortified microgreens displayed a mineral profile with high levels of Se and other minerals, including potassium, calcium, and iron. Sodium selenate treatment, particularly at 40 μM , was most effective in enhancing these mineral contents. This study suggests that sodium selenate is the most efficient inorganic form of Se for the biofortification of Swiss chard microgreens. Further research is needed to evaluate the potential of these Se-enriched microgreens in improving the Se status when consumed as part of a regular diet.

Acknowledgments: The authors would like to thank the Foundation for Science and Technology (FCT) for financial support through national funds FCT/MCTES (PIDDAC): CIMO UIDB/00690/2020 (10.54499/UIDB/00690/2020) and UIDP/00690/2020 (10.54499/UIDP/00690/2020); and SusTEC LA/P/0007/2020 (10.54499/LA/P/0007/2020). National funding by FCT, through the PhD studentship (2023.00954.BD) of A. Pereira. and the research contract (10.54499/CEECINST/00016/2018/CP1505/CT0004) of M.I. Dias.

References:

- [1] G. Genchi, G. Lauria, A. Catalano, M.S. Sinicropi, A. Carocci, IJMS, 24 (2023) 2633.
- [2] A.O. Rataan, S.M. Geary, Y. Zakharia, Y.M. Rustum, A.K. Salem, IJMS, 23 (2022) 2215.
- [3] B.K. Shimada, N. Alfulajj, L.A. Seale, IJMS, 22 (2021) 10713.

Potentially toxic element contamination of European City Soils and Implications for Urban Farming Food Safety

Lourenço Rezende, Joana Bastos Barbosa, Paula Teixeira

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina -
Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
pinto.rezende88@gmail.com

Urban farming has experienced increasing attention in the last decades due to its beneficial impact on the social, economic, and environmental stability of the city. However, the contamination of urban soils with potentially toxic elements (PTE) poses a significant challenge to the safety of urban-grown produce. This study consolidates data from studies on PTE contamination in European urban soils, identifying the risks associated with lead, cadmium, mercury, arsenic, among others.

Through an analysis of data covering 88 locations across 45 cities in 16 European countries, a prevalent pattern of elemental contamination in urban areas has been identified, with over 80% of analyzed locations exhibiting median/mean concentrations of at least one element exceeding national and international farming regulations and/or guidelines.

Notably, hotspots for contamination are found near former and active industrial sites, high-traffic urban centers, and regions affected by long-term atmospheric deposition of pollutants. Lead is the predominant contaminant, with recorded concentrations surpassing established thresholds in over 70% of cases, particularly in sites with a legacy of industrial processing, as is the case of Aubry, Katowice, and Porto, and locations with high-population density such as Berlin, Paris and Seville. Similarly, cadmium exceeds safety limits in 42.8% of cases, while mercury and arsenic concentrations are above guidelines in 18.5% and 35.6% of cases, respectively.

The accumulation of PTE in soil often coincides with the presence of other contaminants, suggesting a common source of pollution or activities contributing to excessive contamination. This interconnected contamination underscores the importance of comprehensive analysis in agricultural soils. Focusing solely on specific sources of pollution or targeted contaminants may lead to overlooking significant concentrations of other unsearched elements. The accumulation of these metals in edible plants poses potential dietary risks, emphasizing the need for risk assessment studies to better understand human exposure through the consumption of urban-grown produce.

Supporting Funds: Financial support for the author Lourenço Pinto de Rezende was provided by the doctoral fellowship 2022.13871.BD

References:

- [1] Sharma K, Basta NT, Grewal PS. Soil heavy metal contamination in residential neighborhoods in post-industrial cities and its potential human exposure risk. *Urban Ecosyst.* 2015;18(1):115-132.
- [2] Ma L, Konter J, Herndon E, et al. Quantifying an early signature of the industrial revolution from lead concentrations and isotopes in soils of Pennsylvania, USA. *Anthropocene.* 2014;7:16-29.
- [3] Nworie OE, Lin C. Seasonal variation in tissue-borne heavy Metal(loid)s in herbaceous plants growing in contaminated soils developed from industrial wastes of industrial revolution age. *Environmental Advances.* 2021;5:100113.

Protein fractions from seaweeds: evaluating fractionation techniques to improve nutritional quality and efficient extraction

Rafaela Nunes¹, Catarina Moreira¹, José A. Teixeira^{1,2}, Cristina M. R. Rocha^{1,2*}

¹ Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

² LABBELS - Associate Laboratory, Guimarães/Braga, Portugal

* cmrocha@ceb.uminho.pt

Seaweeds are reliable substitutes for animal proteins in food applications but protein solubilization and recovery is needed for technological applications, e.g., when the aim is to use proteins as texturizing agents. Moreover, its extraction is usually associated with low extraction yields and/or low protein purities and digestibility, which often leads to the need of additional downstream processes. Also, fractionation techniques can be used in order to improve protein characteristics. In this work, dry fractionation was used to mechanically segregate proteins from celluloses and other fractions based on particle size distribution from different seaweeds (i.e., *G. verrucosa*, *P. palmata*, and *F. vesiculosus*). A first step of milling was used followed by size classification. The effect in the nutritional composition was evaluated and the protein profile of each obtained fraction was determined by several techniques (e.g., gel permeation chromatography (GPC), and FTIR-ATR). Moreover, further fractionation was also studied using the Osborne fractionation method based on a sequential extraction in different solvents (dH₂O, 0.5M NaCl, 70% EtOH, and 0.1 M NaOH).

Results indicated that after milling and size classification, protein increased from the whole to the fine fraction but not in a significant way (from 16.28±0.05 to 18.19±0.07, 11.83±0.18 to 12.47±0.07, and 17.76±0.05 to 20.88±0.08 g_{protein}/100g_{algae} protein for *G. verrucosa*, *P. palmata*, and *F. vesiculosus*, respectively). Also, despite it was observed a clear affinity of proteins for water and alkaline environments, only *P. palmata* showed to have differences in protein extraction between fractions (with no protein extracted in the coarse fraction using dH₂O). On the other hand, different patterns of protein molecular weights were visualized in GPC. Thus, depending on the intended objective, different parameters can be set in order to extract proteins (either with low or high molecular weights) from seaweeds and applied in the development of new food products aiming the reduction of animal proteins use.

Improving the nutritional quality of pea and soybean microgreens with phenolic-enriched nutrient solutions

Matilde Rodrigues^{1,2,3}, M. Beatriz P. Oliveira², Celestino Santos-Buelga³, José Pinela^{1,4,*}

¹ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

² LAQV/REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

³ Grupo de Investigación en Polifenoles (GIP-USAL), Universidad de Salamanca, Facultad de Farmacia, Campus Miguel de Unamuno, E-37007 Salamanca, Espanha

⁴ National Institute for Agricultural and Veterinary Research (INIAV), I.P., Rua dos Lágidos, Lugar da Madalena, 4485-655 Vairão, Vila do Conde, Portugal

* jose.pinela@iniav.pt

Microgreens have emerged as complementary options to enrich and diversify the human diet and combat malnutrition, owing to their high content of micronutrients and bioactive compounds, such as vitamins, mineral elements, and phenolic compounds [1]. Today, the production and characterization of microgreens is an increasingly researched area, with several studies focusing on their agronomic performance and bioactive properties [1,2]. However, the supplementation of plant extracts through the irrigation solution to improve the chemical composition of microgreens is still a relatively underexplored topic. Therefore, this study aims to evaluate the suitability of phenolic-enriched extracts for improving the biochemical composition and bioactive properties of soybean and pea microgreens.

The microgreens will be grown in soilless systems irrigated with an NPK nutrient solution supplemented with varying concentrations of phenolic extracts derived from agricultural crop by-products. The impact of these treatments on seed germination and plant development will be monitored. Following harvesting, morphological attributes will be measured, and the plant samples will be analyzed for their nutritional composition and phytochemical profiles using official methods of food analysis and chromatographic and spectrometric techniques. Additionally, the potential health-promoting effects of the microgreen samples will be assessed *in vitro* through biological activity assays, and simulated gastrointestinal digestion will be performed to evaluate the bioaccessibility of the main phenolic compounds.

This study will contribute to improving the nutritional and bioactive quality attributes of soybean and pea microgreens, while also highlighting the potential of agricultural crop by-products as a valuable source of phenolic compounds. Furthermore, this approach will promote the circularity of food systems, contributing to food security and the promotion of healthy diets.

Acknowledgments: The authors are grateful to the Foundation for Science and Technology (FCT) for financial support through national funds FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (10.54499/UIDB/00690/2020) and UIDP/00690/2020 (10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (10.54499/LA/P/0007/2020); and national funding by FCT, through the individual research grant (2024.04986.BD) of M. Rodrigues.

References:

- [1] A.W. Ebert, Plants, 11 (2022) 571.
- [2] S. Dubey, N. Harbourne, M. Harty, et al., Plants, 13 (2024) 2631.

Assessment of histamine and biogenic amine variability in fish and fishery products: implications for food safety

Manuela M. Moreira^{1,*}, Maria J. Ramalhosa¹, João Couto², Ruben Jorge², Teresa A.C. Oliveira², Ana Cação³, Helga Augusto³, Cristina Delerue-Matos¹

¹ REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

² WeDoTech, 4000-521, Porto, Portugal

³ Cofisa – conservas de peixe da Figueira S.A, terrapleno do porto de pesca - Gala 3090-735 Figueira da Foz, Portugal

* mmdsm@isep.ipp.pt

Aquatic products are highly susceptible to microbial contamination during storage due to their rich nutritional composition, which accelerates spoilage. Some microorganisms in these products produce amino acid decarboxylases, converting free amino acids into biogenic amines (BAs) [1]. Excessive BA consumption can cause food poisoning and other adverse health effects [2]. Among BAs, histamine (HIS) is a primary cause of foodborne illness, while cadaverine (CAD), putrescine (PUT), and tyramine (TYR) can enhance its toxicity [2]. Thus, assessing BA formation in aquatic products is essential to ensure food safety.

This study assessed the presence of eight BAs — tryptamine (TRP), phenylethylamine (PHE), PUT, CAD, HIS, TYR, spermidine (SPD), and spermine (SPM) — in fish and fishery products. High-performance liquid chromatography with fluorescence detection (HPLC-FLD) was used to analyse BA levels in 10 samples: two fresh smaller fish (blue whiting/*verdinho* and Atlantic horse mackerel/*carapau*), four cooked tuna samples (*Thunnus albacares* and *Katsuwonus pelamis* species) and four canned tuna samples (*Katsuwonus pelamis* species) prepared in different mediums (brine, olive oil, extra virgin olive oil and sunflower oil).

Results showed significant variability in BA content across species and product types. In fresh smaller fish, HIS was undetected, but TYR levels were notably high (140–243 mg/kg). *Verdinho* also contained 489 mg/kg of CAD, increasing its toxicological risk. Cooked tuna samples exhibited HIS (up to 26.4 mg/kg) and TYR (up to 143 mg/kg) as dominant amines. In canned tuna, SPM (86.2 mg/kg), SPD (64.7 mg/kg), and HIS (49.1 mg/kg) were the most abundant. Total BA levels were highest in fresh smaller fishes (925–953 mg/kg), followed by cooked tuna (46.3–283 mg/kg) and canned tuna (30.4–230 mg/kg).

These findings point to the potential impact of preparation conditions and fish species on BA formation. Further research on storage conditions, fish species, consumption patterns and exposure assessments are needed to establish safe operating conditions regarding other BA than HIS. Current food safety regulations establish specific limits for HIS in fishery products, particularly for species prone to high HIS formation. However, legal limits for other BAs, such as CAD, PUT, and TYR remain undefined, emphasizing the need for further research to establish safety guidelines.

Acknowledgements: This work has been developed within the scope of “BLUE BIOECONOMY INNOVATION PACT” (Project N°. C644915664-00000026) financed by NextGenerationEU, under the incentive line “Agendas for Business Innovation” of the Recovery and Resilience Plan (PRR). This work also received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Manuela M. Moreira (2023.05993.CEECIND/CP2842/CT0009) is thankful for her contract financed by FCT/MCTES—CEEC Individual Program Contract and to REQUIMTE/ LAQV.

References:

- [1] T. Ding and Y. Li, LWT - Food Science and Technology, 194 (2024) 115793.
- [2] Arulkumar, S. Paramithiotis, and S. Paramasivam, Aquaculture and Fisheries, 8 (2023), 431.

Impact of dry fractionation on the distribution of faba beans' and peas' antinutrients

Elsa Mecha^{1,2*}, Margarida Santos¹, Dejan Knezevic⁴, Sara M. Gaber⁴, Gesine Schmidt⁴, Svein Halvor Knutsen⁴, Maria do Rosário Bronze^{1,2,4}, Teresa Serra^{1,2}, Cátia Saldanha do Carmo¹

¹ Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

² Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal

³ Faculty of Pharmacy, Research Institute for Medicines (iMed.Ulisboa), Universidade de Lisboa, Lisbon, Portugal

⁴ Nofima AS, Norwegian Institute of Food, Fisheries and Aquaculture Research, Aas, Norway

* *emecha@ibet.pt*

Wet fractionation, conventionally used to produce protein concentrates, has a high-cost environmental impact, with a high ecological footprint due to the use of chemicals and water [1]. Dry fractionation based on milling and air classification procedures appears as an alternative sustainable methodology in protein fractionation of pulses and cereals [2]. Nevertheless, the information about the effect of dry-fractionation on bioactive compounds associated with negative effects on nutrient bioavailability, namely the antinutrients is still limited.

This work aimed to assess the impact of dry fractionation on the antinutrient composition of protein- and starch-rich fractions of peas and faba beans. The impact of cooking on the produced fractions was also investigated.

Two varieties of peas (Astronaut and Ingrid) and two varieties of faba beans (Tiffany and Vire) were submitted to dry fractionation using three classifier wheel speeds (5000, 10000, and 15000 rpm). The obtained fractions were analysed regarding their composition in trypsin inhibitors, total saponins and total phenolics using spectrophotometric methods.

Results showed that trypsin inhibitors, saponins and phenolics were concentrated in the fine fraction. Moreover, increasing wheel speed, in general, led to increased concentration of antinutrients in the fine fraction with some particularities, being variety-dependent for trypsin inhibitors and species-dependent in the case of saponins. Starch-rich fractions evidenced a lower concentration of antinutrients for all classifier wheel speeds. The thermal treatment led to a decrease in trypsin inhibitors (up to 57%) and saponins (up to 65%) in the fine fractions.

Considering the development of sustainable protein ingredients for the production of plant-based foods, protein concentrates produced at 10000 rpm complied with the higher protein content requirements and lower content in antinutrients.

The knowledge generated in this work is a step forward, towards the production of protein-rich fractions with improved quality.

Acknowledgments: The authors acknowledge funding received from RCN through GreenPlantFood Project (NFR 319049). The authors acknowledge FCT/MCTES through iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020). The authors acknowledge the PRR through the Agenda VIIAFOOD – Plataforma para a Valorização, Industrialização e Inovação comercial para o Agroalimentar”, framed in the notice nº 02/C05-i01/2022

References:

- [1] M.A.I. Schutyser, P.J.M. Pelgrom, A.J. van der Goot, R.M. Boom, Trends in Food Science & Technology 45 (2015) 327-335.
- [2] C. Saldanha do Carmo, P. Silventoinen, C.T. Nordgård, C. Poudroux, T. Dessev, H. Zobel, U. Holopainen-Mantila, S. Sahlstrøm, S. Halvor Knutsen, J Food Eng (2020) 278.

Fermentation as an approach to improve the nutritional value of grass pea-based food products

Elsa Mecha^{1,2*}, David Santos^{1,3}, Noélia Duarte³, Catarina Prista⁴, Andreia Bento da Silva³, Maria Carlota Vaz Patto², Maria do Rosário Bronze^{1,2,3}

¹ Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

² Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal

³ Faculty of Pharmacy, Research Institute for Medicines (iMed.Ulisboa), Universidade de Lisboa, Lisbon, Portugal

⁴ LEAF (Linking Landscape Environment Agriculture and Food) Research Centre, Instituto Superior de Agronomia, Lisbon, Portugal

* emecha@ibet.pt

Grass pea, *Lathyrus sativus*, is a highly resilient legume that withstand harsh environmental conditions (e.g., drought). This neglected crop is particularly relevant when all other crops, due to unfavorable environmental circumstances, fail to succeed, as an affordable protein source for human nutrition [1,2]. Given the undeniable nutritional value of grass pea, and to diversify the utilization of this NUC (neglected and underutilized crop) in the plant-based food products, innovative processing techniques such as fermentation have been applied.

Within the European DIVINFOOD project, miso and tempeh fermented products, obtained by the action of *Aspergillus oryzae*, *Rhizopus oligosporus* and starter cultures on the boiled grass pea seeds were compared to the previous boiled and raw grass pea seeds in terms of their amino acid, antinutritional (e.g., phenolic compounds, saponins, trypsin inhibitors) contents and volatile composition.

Processing grass pea reduced the existent β -ODAP, β -N-oxalyl-L- α , β -diaminopropionic acid, of raw mature seeds (by 98% and 79% in miso and dehydrated tempeh, respectively), as well as the trypsin inhibitors activity (by 89% and 83%, in miso and dehydrated tempeh, respectively). By contrast, compared to the raw grass pea, the fermented seeds excelled by their higher phenolic content (3.5 times in miso and 2.4 times in dehydrated tempeh), and higher saponin content (4.2 times in miso and 1.8 times in dehydrated tempeh), contributing to enhance the *in vitro* antioxidant activity of the grass pea. The microbial fermentation with release of proteases contributed to increment some amino acids (e.g., methionine and phenylalanine) and subsequent secondary metabolites (saponins and phenolic compounds).

Regarding the volatile composition, the detected compounds of alpha-angelica lactone in miso, and tetramethylpyrazine, in tempeh, associated respectively with nutty and cocoa odor notes, praises the use of fermentation in the development of sensorially attractive grass pea-based products.

Acknowledgments: ADECA – Associação de Desenvolvimento Integrado do Concelho de Alvaiázere. This work was funded by FCT through the Individual Call to Scientific Employment Stimulus - 6th Edition, Reference 2023.07800.CEECIND. H2020 program for funding DIVINFOOD, Co-constructing interactive short and mid-tier food chains to value agrobiodiversity in healthy plant-based food (Grant Agreement n° 101000383).

References:

- [1] K.R. Ramya, K. Tripathi, A. Pandey, S. Barpete, P.G. Gore, A.P. Raina, K.M. Khawar, N. Swain, A. Sarker, Front. Nutr.8 (2022) 826208.
- [2] F. Lambein, S. Travella, Y-H. Kuo, M.V. Montagu, M. Heijde, Planta 250 (2019) 821-838.

Determinant physico-chemical properties to assess technological performance of organic flours

Sérgio Sousa^{1,*}, Ema Dias², Ana Gomes¹

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, 4169-005 Porto, Portugal

² Germen – Moagem de Cereais S.A., Rua Joaquim Pinto, 91, 4460-339 Matosinhos, Portugal

* sdsousa@ucp.pt

It is well known that the physico-chemical properties of cereal flours are directly correlated with their technological performance and ultimately determine their potential utilization in distinct products. This study aimed to characterize organic flours derived from three different cereals—wheat, rye, and spelt. These cereals were chosen due to their ability to thrive in Portugal's favourable climatic conditions.

The physico-chemical properties of each organic cereal flour, were evaluated using various methodologies and technologies. These included the falling number test for enzyme activity, Mastersizer for particle size distribution, and scanning electron microscopy for size and morphological analysis, among others.

The results indicated that among the three flours, dough made from wheat flour was expected to be firmer compared to those prepared with rye or spelt flour, since the flour revealed the lowest α -amylase enzymatic activity (highest falling number, 350). Regarding particle size distribution, wheat and rye flours displayed a bimodal distribution, whereas spelt flour followed a normal distribution pattern. A primary peak was observed in all flours within the 20–30 μm range, while a secondary peak appeared at 60 μm for wheat and 135 μm for rye. These findings may have an important impact on the flours water absorption capacity and, consequently, on the texture of the final product. In turn, X-ray diffraction analysis provided insights into the crystallographic structure, revealing that rye flour exhibited the lowest crystallinity and hence is expected to be the most susceptible to enzymatic hydrolysis, leading to higher digestibility. Differential scanning calorimetry demonstrated that rye flour presented the lowest retrogradation tendency, which contributes to slower staling, resulting in prolonged freshness.

Ultimately, understanding the physico-chemical properties of each organic cereal flour is essential and should be carefully considered when selecting the most suitable option for developing a specific food product.

Acknowledgments: This work was developed under the framework of project Agenda VIIAFOOD – Platform for Valorisation, Industrialization and Commercial Innovation for the Agri-Food Industry”, financed through the Program for Recovery and Resilience (PRR). The authors would like to acknowledge GERMEN – Moagem de Cereais S.A, for their support in carrying out the project, and the scientific collaboration under the FCT project UIDB/50016/2020.

Efficient and scalable spray-drying of encapsulated oleoresins for sustainable ingredient production

Gabriel Mota Ribeiro¹, Christopher Rafael^{1*}, Beatriz Fernandes^{2,3}, Carmo Serrano^{2,4}

¹FMSI, Lda, Rio de Mouro, Portugal

²INIAV, National Institute for Agrarian and Veterinary Research, Oeiras, Portugal

³CERENA, DEQ, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal

⁴LEAF- Linking Landscape, Environment, Agriculture and Food Research Unit, Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

*c.rafael@fmsi.info

Oleoresins, valued for their concentrated flavours and high bioactive compounds content [1], hold significant potential in the food market. Encapsulation enhances their stability, functionality, and shelf life [2], with spray drying being a widely used technique for producing stable powdered ingredients [3]. However, conventional spray drying processes requires high energy inputs — especially during scale-up — posing economic and environmental challenges [4].

To address this, we optimized the spray drying process by testing different oleoresin and encapsulating agent-to-water ratios (solid ratios) to maximize encapsulation efficiency and product stability while reducing production costs, processing time, and carbon footprint.

In bench-to-pilot trials (0.5 kg of feed mixture), with solid ratios ranging from 13% and 39% — while maintaining constant inlet temperature and pump speed—total product yields increased from 31.80 g to 68.60 g. The optimal solid ratio was 26%, yielding 58.90 g of product, with the lowest water activity ($A_w = 0.25$) and humidity (2.45%) and a high encapsulation efficiency of 87.89%. At this ratio, 45.31% of total solids were successfully recovered post-drying.

Compared to the previously used 13% solid ratio, this optimized approach halved drying time while maintaining similar final product yields. The 26% solid ratio presents lower A_w and humidity levels, which indicate more efficient drying. This solid ratio improved industrial feasibility by increasing productivity, reducing energy consumption, and lowering the carbon footprint — all while preserving product quality.

Acknowledgments: The authors would like to acknowledge FCT for supporting the project “Pacto da Bioeconomia Azul” (PRR-C644915664-00000026).

References:

- [1] C. Serrano, M. Sapata, D. Castelo-Branco, A. Tasso, A. Marques, C. Viegas, D. Figueira, N. Komora, The influence of salt reduction with encapsulated oleoresins on the quality of mayonnaise, mustard, and ketchup, *Frontiers in Nutrition*, 11 (2024).
- [2] B. Fernandes, M. C. Oliveira, A. C. Marques, R. G. Dos Santos, C. Serrano, Microencapsulation of essential oils and oleoresins: Applications in food products, *Foods*, 13(23) (2024), 3873.
- [3] N.K. Mohammed, C.P. Tan, Y.A. Manap, B.J. Muhialdin, A.S.M. Hussin, Spray Drying for the Encapsulation of Oils—A Review, *Molecules*, 25 (2020), 3873.
- [4] C.I. Piñón-Balderrama, C. Leyva-Porras, Y. Terán-Figueroa, V. Espinosa-Solís, C. Álvarez-Salas, M.Z. Saavedra-Leos, Encapsulation of Active Ingredients in Food Industry by Spray-Drying and Nano Spray- Drying Technologies, *Processes*, 8 (2020), 889.

Incorporation of *Tenebrio molitor* flour in olive pâtés and almond butters: A sustainable and nutritional alternative

Maria Ramos^{1,*}, Inês Pedro¹, Norton Komora², Patrícia Araújo^{1,2}

¹ Casa Mendes Gonçalves, 2150-268, Golegã, Portugal

² LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017, Lisbon, Portugal

*maria.ramos@casamg.pt

The growing consumer awareness of nutritional and environmental aspects has driven the market to adopt sustainable food solutions. One emerging trend is the incorporation of edible insects, particularly for their high protein and mineral content and lower environmental impact. However, cultural and psychological barriers still hinder acceptance in Europe [1]. To overcome this, insects are introduced as flour in products that mask their taste and appearance. This study examines the incorporation of *Tenebrio molitor* flour in olive pâtés and almond butters.

The *T. molitor* flour used in this study was supplied by Thunder Foods. Olive pâtés were developed with 3%, 6%, and 9% *T. molitor* flour, and analyzed for physicochemical, nutritional, microbiological, and sensory properties. A control sample was used for comparison. Results showed the 3% insect flour sample was most preferred, with 50% of the tasting panel willing to purchase it. Microbiological tests indicated no microbial growth after 60 days, and nutritional analysis confirmed increased protein content, from 1.9% (control) to 3.1% (3% sample). For almond butters, formulations with 5% and 10% of *T. molitor* flour were tested. The 5% sample showed no significant difference compared to the control and was the most accepted. It also demonstrated improved protein content, from 21.4% (control) to 22.9% (5% sample).

These findings suggest that *Tenebrio molitor* flour can enhance nutritional value in products while minimizing consumer resistance. Future studies should optimize formulations, explore consumer acceptability, and assess large-scale production feasibility to integrate insect-derived ingredients into the market successfully.

Acknowledgments: This work was supported by InsectERA Mobilizing Agenda, nº C644917393-00000032, C05- i01 in PT Recovery and Resilience Plan.

References:

[1] Ribeiro, J. C., Santos, C., Lima, R. C., Pintado, M. E., & Cunha, L. M. (2022). Impact of defatting and drying methods on the overall liking and sensory profile of a cereal bar incorporating edible insect species. DOI:10.1016/j.fufo.2022.100190

Incorporation of *Acheta domesticus* powder in sauces: A sustainable and nutritional alternative

Maria Ramos^{1,*}, Inês Pedro¹, Ema Camacho², Norton Komora³

¹Casa Mendes Gonçalves, 2150-268, Golegã, Portugal

²Universidade Católica Portuguesa CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal ³ LEAF – Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017, Lisbon, Portugal

* maria.amos@casamg.pt

The increasing awareness of sustainability and nutritional benefits has led to the exploration of edible insect incorporation into food products. Mainly, products that can minimize their flavour, such as sauces. This study investigates the integration of *Acheta domesticus* (house cricket) powder in chipotle sauces, comparing three formulations: standard sauce, sauce with egg yolk and *A. domesticus* and sauce with modified starch and *A. domesticus*. The house cricket powder used in this study was supplied by Cricket Farming Co. Physicochemical, nutritional, microbiological, sensory, and microstructural analyses were conducted.

Results indicated that the sauce with egg yolk and *A. domesticus* was the most similar to the standard in terms of oil droplet microstructure and color, with minimal visible differences ($\Delta E = 10.65$). Sensory analysis showed comparable acceptance levels, with 50% of the panel indicating they would likely purchase the product with egg yolk and *A. domesticus*. Nutritionally, the sauce with egg yolk and *Acheta domesticus* had higher protein content than the standard formulation while maintaining similar viscosity, 28067 ± 6762^a for the standard one and 27767 ± 2040^a for the one with egg yolk and *A. domesticus*.

These findings highlight the potential of using *Acheta domesticus* in sauces without significantly altering sensory or structural properties. Future research should focus on optimizing formulations, expanding consumer acceptability studies, and conducting shelf-life testing to evaluate product stability over time. This study contributes to the development of sustainable and nutritious food alternatives, addressing both environmental and consumer acceptance challenges.

Acknowledgments: This work was supported by InsectERA Mobilizing Agenda, nº C644917393-00000032, C05- i01 in PT Recovery and Resilience Plan.

Exploring the effect of feeding and production batches on the microbiological safety of *Tenebrio molitor* larvae

Teresa Bento de Carvalho¹, Inês Pereira¹, Joana Bastos Barbosa¹, Tiago Ribeiro², Vasco Esteves², Paula Teixeira¹

¹Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina -
Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

²Tecmafoods - Insect Based Feed & Food, Lda – Rua Sousa Prata 678 Armazém B, Leça do
Balio, Portugal
jbarbosa@ucp.pt

Nutrition is essential for human survival, but unsustainable food production methods and eating habits can have a negative effect on the environment [1]. Edible insects, particularly *Tenebrio molitor* larvae, are gaining popularity in Western countries as an alternative to animal proteins like meat, poultry, and dairy due to their potential to provide sustainable protein while requiring fewer resources such as land, water, and contributing fewer greenhouse gas emissions [2]. This has sparked increased research into their food safety. This study aimed to assess the microbiological quality of fresh *T. molitor* larvae subjected to different feeding regimes (water, potato, carrot, vegetable mix) and sourced from three separate production batches. Various microbiological parameters were evaluated, including Total Viable Counts (TVC), lactic acid bacteria (LAB), enterococci, *Enterobacteriaceae*, *Bacillus cereus*, *Staphylococcus* spp., *Escherichia coli*, aerobic and anaerobic bacilli spores, yeasts, moulds, and the detection of *Salmonella* spp. and *Listeria monocytogenes*. The results showed no significant differences in microbiological parameters across the production batches for any of the diets. Larvae fed with potatoes exhibited slightly higher microbial loads, especially for TVC, LAB, and *Enterobacteriaceae*. Neither *Salmonella* spp. nor *L. monocytogenes* were detected in any of the samples. These findings suggest that fresh mealworms possess inherent qualities that lead to higher microbial loads, particularly in TVC. Proper hygiene during rearing is crucial for insect quality, and the consistency of the results across batches indicates compliance with hygiene standards, feed quality, and rearing conditions, all of which are key to ensuring food safety. Further research, including testing additional batches from different seasons, is recommended to explore the influence of external factors on the microbiological safety of mealworms for human consumption.

Acknowledgments: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project INSECTSAFE - Protein for tomorrow: Exploring the safety of edible insects for global food demands (<https://doi.org/10.54499/2023.12117.PEX>). Financial support for author T. Bento de Carvalho was provided by a doctoral fellowship 2023.03709.BD (FCT).

References:

- [1] M. Gibas-Dorna, W. Żukiewicz-Sobczak. Sustainable Nutrition and Human Health as Part of Sustainable Development. *Nutrients*, Jan 10;16(2024):225. <https://doi.org/10.3390/nu16020225>
- [2] M. Li, C. Cao, X. Li, L. Jiang, W. Zhang, M. Li, H. Liu, Y. Fang, S. Liu, G. Yang, & X. Hou. Edible Insects: A New Sustainable Nutritional Resource Worth Promoting. *Foods*, 12(2023), 4073. <https://doi.org/10.3390/foods12224073>

Formation of *Acinetobacter* biofilms on stainless steel: risks for food safety and industrial contamination

Marta Carvalho*, Joana Barbosa and Paula Teixeira

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina e
Laboratório Associado, Escola Superior de Biotecnologia.

*s-mipcarvalho@ucp.pt

The genus *Acinetobacter* is widely distributed in natural and artificial environments and is concerning due to its resistance to antibiotics and disinfectants. Contamination of food, particularly meat, dairy, and vegetables, can occur during processing and handling. *Acinetobacter* biofilms pose a significant threat to food safety by forming resilient bacterial communities on food contact surfaces like stainless steel and plastic, increasing resistance to cleaning and sanitization. This study evaluates the biofilm formation capacity of 20 *Acinetobacter* strains isolated from food on stainless steel surfaces. Each strain was inoculated in Tryptic Soy Broth (TSB) with stainless steel coupons and incubated at 22 °C for five days. Biofilms were collected by swabbing, followed by vortexing. Serial decimal dilutions were performed in Phosphate Buffered Saline (PBS) and inoculated onto Tryptic Soy Agar (TSA) plates for colony counting. Three independent experiments were conducted, including controls.

Results showed that *A. baumannii* 135.2 (Log CFU = 5.6), *A. calcoaceticus* 109.1 (Log CFU = 5.8), and *A. pittii* 87.2 (Log CFU = 5.3) exhibited the highest biofilm production. In contrast, *A. johnsonii* 145.3 (Log CFU = 4.2), *A. baumannii* 133.1 (Log CFU = 4.4), and *A. johnsonii* 118.9 (Log CFU = 4.6) had the lowest biofilm formation. Significant variability was observed, even within the same species, suggesting that genetic differences or environmental factors influence biofilm development.

These findings emphasize the importance of preventing and controlling *Acinetobacter* biofilms to ensure food safety and reduce risks associated with their persistence in the food production chain.



Impact of *Escherichia coli* on the Growth of *Listeria monocytogenes*: A Preliminary Assessment

Mónica Oliveira, Joana Barbosa, Paula Teixeira*

Universidade Católica Portuguesa, CBQF-Centro de Biotecnologia e Química Fina Laboratório
Associado, Escola Superior de Biotecnologia, Porto, Portugal

* pcteixeira@ucp.pt

Listeria monocytogenes is a foodborne pathogen responsible for listeriosis, a life-threatening illness that poses a significant risk to susceptible populations, including the elderly, pregnant women, neonates, and immunocompromised individuals [1]. The human digestive system presents multiple barriers to *L. monocytogenes*, including the gut microbiota, which plays an important role in limiting bacterial survival and infection potential [2].

This study aimed to investigate the interaction between *L. monocytogenes* and *Escherichia coli*, a key member of the human gut microbiota, by analyzing their growth dynamics both independently and in co- culture. Two *L. monocytogenes* strains (2542 and 2606, serotype 4b) were cultured alone and with *E. coli* over a 26-hour period, with samples collected every two hours for enumeration on selective media.

Growth analysis revealed that *E. coli* maintained comparable proliferation patterns regardless of whether it was cultured alone or with *L. monocytogenes*. However, *L. monocytogenes* exhibited distinct responses depending on the strain. During the first 12 hours, both strains followed similar growth patterns in co-culture and single culture. Beyond this point, strain 2542 displayed a 1.8 log cycles reduction when co-cultured with *E. coli*, whereas strain 2606 showed a more pronounced decrease of 2.1 log cycles.

These findings suggest that strain 2606 is more susceptible to the presence of *E. coli* than strain 2542, highlighting potential strain-specific interactions within the gut environment. Future studies will expand on these observations by incorporating additional strains and examining their behavior in the presence of other predominant gut bacteria to better understand the impact of microbial interactions on *L. monocytogenes* survival.

Acknowledgements: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project GenoPhenoTraits4Persistence - Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment (PTDC/BAA-AGR/4194/2021); <https://doi.org/10.54499/PTDC/BAA-AGR/4194/2021>. The authors would like to thank the scientific collaboration under the Fundação para a Ciência e a Tecnologia (FCT) project UIDP/00329/2020. Financial support for author Mónica Oliveira was provided by the doctoral fellowships 2021.08345.BD.

References:

- [1] Swaminathan, B., & Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. *Microbes and Infection*, 9(10), 1236–1243. <https://doi.org/10.1016/j.micinf.2007.05.011>
- [2] Bagatella, S., Tavares-Gomes, L., & Oevermann, A. (2022). *Listeria monocytogenes* at the interface between ruminants and humans: A comparative pathology and pathogenesis review. *Veterinary Pathology*, 59(2), 186–210. <https://doi.org/10.1177/03009858211052659>

Agile methodologies used to design healthy and sustainable food in SMEs in Iberoamerica

Jairo Torres^{1,*}, Letricia Barbosa-Pereira¹, Raquel Sendón¹, Joao Lima²

¹ FoodChemPack Research Group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Campus Vida, 15782 Santiago de Compostela, Spain,

² H&TRC - Health & Technology Research Center, Coimbra Health School, Polytechnic University of Coimbra, Coimbra, Portugal.

* jairoalonso.torres@rai.usc.es

Introduction: The design and formulation of food, a complex and time consuming process, faces challenges in SMEs, where it often relies on trial and error, limiting its competitiveness in the market. Given the need for agile and sustainable solutions, this research seeks to identify the current methodologies used in the development of food products in SMEs to identify opportunities for improvement and innovation.

Objective: To identify the agile methodologies and software used to design healthy and sustainable food in SMEs in Iberoamerica.

Method: An ethnographic study was conducted in Latin American countries (Brazil, Colombia, Chile, Mexico, and Uruguay) and European country (Portugal), through semi-structured interviews with food design managers, based on a questionnaire of 6 questions with a maximum duration of 30 minutes. Additionally, the AEIOU non-participant observation method was applied through visits to SMEs or analysis of visual materials.

Results preliminaries: The sample included, food engineers, chefs, electronic engineers, nutritionists, and administrators, belonging to multinational companies, medium to large companies, and SMEs, covering food additives, dairy, meats, soups, and other products. The majority indicated that the food design process takes approximately 1 to 2 years. The main limitations identified were the lack of availability of raw materials, high production costs, limited knowledge of regulations, lack of clear guidelines, costly processes, cultural barriers, and the need for subsistence-based production. Regarding the knowledge of agile methodologies, SMEs indicated a lack of knowledge about methodologies and software; medium-sized companies only mentioned the use of the Stage-Gate method, while large companies reported having internal standardized methods, without revealing details due to corporate policies. In the non-participant observation, it was found that SMEs lack sophisticated equipment and software assistance for food design.

Conclusion: The data collected from this preliminary estudy indicated that SMEs in Iberoamerica show limited use of agile methodologies and software to design healthy and sustainable food.

Acknowledgments: This research was funded the “Consellería de Educación, Ciencia, Universidades e Formación Profesional, Xunta de Galicia”, within the project “Consolidación y estructuración 2023—Grupos de potencial crecimiento (GPC)” (Ref. ED431B2023/52).

References:

- [1] Bigliardi B, Filippelli S. A review of the literature on innovation in the agrofood industry: sustainability, smartness and health. *Eur J Innov Manag.* 2022;25(6):589–611.
- [2] Garcia-Garcia G, Azanedo L, Rahimifard S. Embedding sustainability analysis in new food product development. *Trends Food Sci Technol.* 2021;108(December 2020):236–44.
- [3] Lays Karolina Ferreira Salviano; Doriane Braga Nunes. *Inovação No Setor Alimentício : Estratégias Competitivas E Tecnológicas Innovation in the Food Sector : Sect Innov Food Strateg Compet.* 2017.
- [4] Sijtsema SJ, Snoek HM. Involving consumers in food product development: Perspectives on the application of circular food design. *Front Sustain Food Syst.* 2023;7.
- [5] Lisitsyn AB, Chernukha IM, Nikitina MA. Russian methodology for designing multicomponent foods in retrospect. *Foods Raw Mater.* 2020;8(1):2–11.

Quality and microbial profile of chourição: influence of salt reduction

**Joana Bastos Barbosa¹, Teresa Bento de Carvalho¹, Maria Alexandra Azevedo²,
Fátima Carvalho³, Paula Teixeira¹**

¹Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina -
Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

²Colab4Food – Collaborative Laboratory for innovation in the Agri-food Industry, Vila do Conde,
Portugal

³R&D Department, Primor Charcutaria Prima – S.A., Avenida Santiago de Gavião, nº 1142,
Gavião, 4760-003, Vila Nova de Famalicão, Portugal
jbarbosa@ucp.pt

Salt has been used for centuries to preserve meat products. Since excessive sodium consumption is linked to cardiovascular conditions, reduced-salt versions of traditional meat products like chourição have been introduced. While lowering salt levels helps to address excessive sodium intake, it brings up concerns about the microbiological safety of these products, as it may promote the growth of harmful microorganisms.

The purpose of this study was to evaluate the impact of salt reduction in the formulation of sliced chourição on total viable counts (TVC) and lactic acid bacteria (LAB) counts, in order to evaluate its shelf life, and also to study the survival of *Listeria monocytogenes* in these artificially contaminated products stored at 4 and 10 °C.

Microbiological analysis were conducted on both standard and 25% (w/w) salt-reduced chourição slices stored at 4 °C and 10 °C (abuse temperature), throughout the 60 days storage period. Slices of both standard and salt-reduced chourição were inoculated with a cocktail of seven *L. monocytogenes* strains (~3 log CFU/g) and stored at the same conditions.

An increase in the number of LAB and TVC was observed during the 60 days of storage at both tested temperatures, although this increase was more pronounced at 10 °C. Regarding LAB, an increase of 4 and 5 log cycles was recorded at 4 °C and 10 °C, respectively, for the standard chourição, while a 5 log cycles increase was observed at both temperatures for the salt-reduced product. For TVC, a 4 and 6-log cycle increase was observed at 4 °C and 10 °C, respectively, in the standard formulation, while the novel prototype showed a 6 and 7-log cycle increase. The salt-reduced versions showed a more pronounced bacterial increase compared to the standard samples, especially during storage at 10 °C. Regarding *L. monocytogenes* contamination, no visible differences were found between standard and salt-reduced chourição. However, as anticipated, the pathogen was able to proliferate more rapidly in the product when stored at 10 °C, with an increase of around 5 log cycles after 30 days, in contrast to the increase of around 1 to 2 log cycles observed at 4 °C over the same time frame.

Salt is essential for preserving meat products. Therefore, any product reformulation involving salt reduction must ensure similar economic and technological feasibility and, most critically, preserve the microbiological safety of the final product.

Acknowledgments: This work was supported by agenda VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação Comercial para o setor Agroalimentar (C644929456-00000040), funded by PRR – Plano de Recuperação e Resiliência e pelos Fundos Europeus NextGeneration EU Financial. Financial support for author T. Bento de Carvalho was provided by a doctoral fellowship 2023.03709.BD (FCT).

References:

- [1] Lücke, F.-K. (2000). Fermented meat products. *Food Research International*, 27(3), 299-307. [https://doi.org/10.1016/0963-9969\(94\)90098-1](https://doi.org/10.1016/0963-9969(94)90098-1)
- [2] He, F. J., & MacGregor, G. A. (2009). A comprehensive review on salt and health, and the role of salt reduction in preventing cardiovascular disease. *Journal of Human Hypertension*, 23(6), 363-384. <https://doi.org/10.1038/jhh.2008.144>

Making *garum* in Troia in the 21st century

Sales, Joana^{1*}, Vicente, Victor², Almeida, Pedro², Vaz Pinto, Inês³, Raymundo, Anabela¹, Prista, Catarina¹

¹ LEAF–Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

² Selo de Mar, Setúbal, Portugal

³ Troia Resort / CEAACP - Universidade de Coimbra

* joanasales@isa.ulisboa.pt

Marine resources such as fish and molluscs are considered raw materials with great nutritive potential, much appreciated by Portuguese consumers. *Garum*, frequently cited in classical texts, was a fermented sauce made from fish and salt which had an important place in Greeks and Romans daily life [1]. It was considered a noble salty sauce, used by higher society classes as a flavour enhancer and a healthy replacement for salt in all kinds of dishes.

In 2021, the teams from the Selo do Mar and Roman Ruins of Tróia project decided to return to making *Garum* in a salting tank at this archaeological site, using sardine (*Sardina pilchardus*), the commonly used fish in Tróia and Lusitânia in Antiquity [2].

Garum was produced in one of the smallest Roman tanks (1.35 m³), placing sardines cut into pieces, salt and water in a food-grade certified polypropylene bag, placed inside the salting tank absorbing the heat from the walls, keeping the temperature relatively constant over the months. The mixture was prepared and let to ferment for around five months. *Garum* was stirred frequently, and each time samples were collected and kept for nutritional and microbiological analysis.

The results confirm that the *garum* produced is a stable and safe product for consumption and demonstrate that it is an important source of iron, potassium, calcium, magnesium, phosphorus and sulfur, with significant increases over the months of maturation, and consequently with an increase in inorganic matter. Protein content reached its maximum level after 3 months of maturation, followed by a significant decrease. The decrease could be related to the degradation of free amino acids and consequently a decrease in the total nitrogen content. However, this degradation could have a positive impact on the formation of characteristic aromas.

Acknowledgments: This work was supported by *Fundação para a Ciência e a Tecnologia* (FCT) through the strategic project UID/AGR/04129/2020 (LEAF) and by Selo de Mar. We also thank the support of the Roman Ruins of Tróia, Troia Resort.

References:

- [1] R.I. Curtis. *Garum and salsamenta. Production and commerce in materia medica*. E. J. Brill, Leiden. (1991).
- [2] I.V. Pinto, A.P. Magalhães, P. Brum, M. Figueiredo, F. Santos, S. Gabriel. "Uma sepultura de mesa na Ponta do Verde (Tróia)", *Do Período Romano Imperial à Idade Contemporânea. Actas do IX Encontro de Arqueologia do Sudoeste Peninsular*, vol. 2 (I. V. Pinto, Rosa V. Gomes e J. A. Pérez Macías, coords.), DIGITAR – Digital Journal of Archaeology, Architecture and Arts (2022) 8 p. 102-121.

Impact of Refrigeration on the viability of *Campylobacter* spp. in water

Ana Rita Barata^{1,2,3}, Beatriz Ferreira^{1,4}, Patrícia Oliveira¹, Hugo Guedes¹, Maria José Saavedra^{2,3}, Gonçalo Almeida^{1,5*}

¹National Institute for Agricultural and Veterinary Research (INIAV) I.P., Vila do Conde, Portugal

²Center for Research and Agro-environmental and Biological Technologies (CITAB) and Inov4Agro, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

³Center for Animal and Veterinary Science (CECAV), Associated Laboratory of Animal and Veterinary Science (AL4Animals), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

⁴Faculty of Pharmacy, University of Porto (FFUP), Porto, Portugal

⁵Center for Animal Science Studies (CECA-ICETA), Associated Laboratory of Animal and Veterinary Science (AL4Animals), Porto, Portugal

* goncalo.almeida@iniav.pt

Campylobacter spp. is the leading causes of bacterial gastroenteritis in humans, with infections often transmitted through contaminated food, such as poultry meat [1]. Despite their high incidence, cases of campylobacteriosis are frequently underreported, largely due to the difficulty of culturing *Campylobacter* in conventional laboratories [2]. The bacteria require specific growth conditions, such as a microaerophilic atmosphere and high incubation temperatures, making their detection more challenging compared to other bacterial pathogens. Furthermore, the choice of culture medium can significantly influence the success of *Campylobacter* growth, meaning that the effectiveness of different media can impact the ability of laboratories to accurately identify the bacteria [3].

This study aimed to evaluate the survival of *Campylobacter jejuni* and *Campylobacter coli* in water stored at 4°C over time. Bacterial strains were inoculated into sterile water and incubated at refrigeration temperature. Enumeration was performed at regular intervals using three selective culture media to assess bacterial viability and efficacy of each culture medium, with the goal of determining which medium supports the best bacterial rate.

The results showed a gradual decline in viable *Campylobacter* cells, with a survival period exceeding 48 hours. As usual, both species demonstrated the ability to persist for long periods at 4°C, suggesting that ice water may act as a temporary reservoir for *Campylobacter* survival. In relation to the culture media, it was also possible to observe that there are differences in effectiveness and that different culture media can demonstrate different survival rates.

In conclusion, while refrigeration reduces *Campylobacter* viability, the bacteria can persist long enough to pose a contamination risk. These findings highlight the importance of monitoring water sources and implementing control measures to minimize the risk of *Campylobacter* transmission through water.

Acknowledgments: Ana Rita Barata would like to thank the Foundation for Science and Technology (FCT I.P.) for the doctoral scholarship ref. 2023.04257.BDANA. This work is supported by National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UI/04033 And LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>).

References:

- [1] Z. Bukari, T. Emmanuel, J. Woodward, R. Ferguson, M. Ezughara, N Darga, B. Lopes, Trop. Med. Infect. Dis. 10 (2025) 25.
- [2] Khairullah, S. Yanestria, M. Effendi, I. Moses, M. Kusala, K. Fauzia, S. Ayuti, I. Fauziah, O. Silaen, P. Riwu, S. Aryaloka, F. Dameanti, R. Raissa, A. Hasib, A. Furqoni, Open Vet J. 14 (2024) 8.
- [3] J. Lagier, S. Edouard, I. Pagnier, O. Mediannikov, M. Drancourt, D. Raoult. Clin Microbiol Rev. 28 (2015).

Cold Survival: Does strain origin affect the growth of *Listeria monocytogenes* in milk at storage temperatures?

Ana Rita Barata^{1,2,3}, Beatriz Ferreira^{1,4}, Inês Soares¹, Patrícia Oliveira¹, Hugo Guedes¹, Gonçalo Almeida^{1,5*}

¹National Institute for Agricultural and Veterinary Research (INIAV) I.P., Vila do Conde, Portugal

²Center for Research and Agro-environmental and Biological Technologies (CITAB) and Inov4Agro, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

³Center for Animal and Veterinary Science (CECAV), Associated Laboratory of Animal and Veterinary Science (AL4Animals), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

⁴Faculty of Pharmacy, University of Porto (FFUP), Porto, Portugal

⁵Center for Animal Science Studies (CECA-ICETA), Associated Laboratory of Animal and Veterinary Science (AL4Animals), Porto, Portugal

* goncalo.almeida@iniav.pt

Listeria monocytogenes is a foodborne pathogen responsible for listeriosis, a potentially fatal infection, particularly in vulnerable populations such as pregnant women, the elderly, and immunocompromised individuals [1]. The bacterium can grow at low temperatures, including refrigeration, allowing it to proliferate in foods like dairy products, which are often considered safe at such temperatures [2]. Milk provides an ideal medium for *L. monocytogenes*, making it a potential vehicle for human transmission [3]. However, limited information exists on how different strains behave under refrigeration.

The aim of this study was to evaluate the growth at 4°C of 12 strains of *Listeria monocytogenes* from different sources in three different types of milk: raw, thermized, and pasteurized milk samples. The study sought to determine whether the origin of the strains influences their survival and proliferation under refrigerated conditions, which is crucial to understanding the potential risks in dairy products and trying to understand which type of milk could be the greatest growth enhancer.

Milk samples were inoculated with *L. monocytogenes* to reach an initial concentration of 10³ CFU/mL. All milk samples were incubated at refrigeration temperatures 4 ± 2°C. Enumeration of *L. monocytogenes* was performed according to (ISO 11290-2:2017, 2017).

The results did not indicate an effect of strain origin on growth rates. All strains exhibited similar growth patterns, suggesting that the origin of the strains does not affect their ability to proliferate in refrigerated environments. However, all strains exhibited a higher growth rate in pasteurized milk when compared with the other two types of milk.

This highlights the continuous risk of contamination in dairy products under refrigeration and underscore the need for effective control measures to mitigate the risk of *L. monocytogenes* contamination in dairy products.

Acknowledgments: The authors would like to thank to the ongoing project GenoPheno4trait (PTDC/BAA-AGR/4194/2021), funded by FCT.

References:

- [1] G. Almeida, R. Magalhães, L. Carneiro, I. Santos, J. Silva, V. Ferreira, T. Hogg, P. Teixeira, International Journal of Food Microbiology, 167 (2013) 3.
- [2] V. Lappi, A. Ho, K. Gall, M. Wiedmann, Journal of Food Protection, 67 (2004) 5.
- [3] M. Vilar, E. Yus, M. Sanjuán, F. Diéguez, J. Rodríguez-Otero, Journal of Dairy Science, 90 (2007) 11.

Consumers' acceptance and conceptualisation of innovative food products with edible algae

Inês Magalhães¹, Sofia Sousa², Tânia Calçada³, Armando Perez-Cueto⁴, Luís M. Cunha^{2*}

¹ MSc in Consumer Sciences and Nutrition, Faculty of Sciences, University of Porto, Porto, Portugal

² GreenUPorto/Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, 4485-646 Vila do Conde, Portugal

³ SONAE MC, 4464-501 Matosinhos, Portugal

⁴ Department of Food, Nutrition and Culinary Science, University of Umeå, Sweden

* lmcunha@fc.up.pt

Algae represent a sustainable food source, but their adoption in daily diets depends on consumers' acceptance [1]. However, information about the Portuguese consumers' perceptions of algae-based products and associations with relevant determinants is still scarce. This study investigated the conceptualisation and willingness among Portuguese adults to eat products with edible algae.

A web-based questionnaire was applied. A free-word association methodology [2] and the Food Action Rating (FACT) scale [3] were used to assess conceptualisation and acceptance/willingness to eat. A cluster analysis was conducted to identify different consumer segments. Frequencies of the mentions for dimensions were computed. Correspondence analysis between consumer segments was made. Descriptive statistics were applied to socio-demographic characteristics. The Friedman test was used to determine the differences in the acceptance of different food categories containing edible algae. Factorial analysis was applied to the FACT scale results.

Participants (n=494) were grouped into four segments: Moderate Critics (n=123), Rejecters (n=116), Moderate Admirers (n=182) and Acceptors (n=73). To conceptualise "algae", "Nature" was the most cited dimension. To "algae" and "food", "Food" was the most mentioned dimension. Pasta (FACT – 5.6 ± 1.9) and dry seasonings (FACT – 5.6 ± 1.9) were the product categories with the highest acceptance levels. Gender, level of education, marital status, number of household members, financial situation, occupation, diet, healthiness of the diet, consumption of ethnic foods, familiarity, food neophobia, green consumption values and trust showed significant associations with acceptance.

This study can offer valuable insights to the food industry in developing novel products that respond to consumer preferences and needs, contributing to introducing new, sustainable, and nutrient-rich algae products in the agri-food market.

Acknowledgments: Authors Magalhães, Sousa and Cunha acknowledge support from the strategic project UID 05748: GreenUPorto - *Centro de investigação em Produção Agroalimentar Sustentável*, funded by national funds via FCT (Foundation for Science and Technology - Portuguese Ministry of Science, Technology and Higher Education). The authors acknowledge Inês Magalhães internship at the University of Umeå, with the support of the Erasmus+ programme of the European Union.

References:

- [1] Diaz, C. J., Douglas, K. J., Kang, K., Kolarik, A. L., Malinovski, R., Torres-Tiji, Y., Molino, J. V., Badary, A., & Mayfield, S. P. (2023). Developing algae as a sustainable food source. *Frontiers in nutrition*, 9, 3147.
- [2] Sousa, P. M. d., Moreira, M. J., Moura, A. P. d., & Cunha, L. M. (2021). Conceptualização e perspetivas do consumidor face à Economia Circular na valorização de novos produtos/ingredientes em contexto alimentar. *Captar: ciência e ambiente para todos*.
- [3] Schutz, H. G. (1965). Food action rating scale for measuring food acceptance. *Journal of Food Science*, 30(2), 365-374.

Encapsulated aromatic oleoresins and KCl as NaCl replacements in meat products

Patrícia Bernardo^{1,2}, Maria J. Fernandes^{1,2}, Maria H. Fernandes^{1,2}, Maria P. Teixeira^{1,2}, Carmo Serrano^{3,4}, Luís Patarata^{2,5} and Maria J. Fraqueza^{1,2*}

¹CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

²AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences

³INIAV - National Institute for Agrarian and Veterinary Research, Oeiras, Portugal

⁴LEAF - Linking Landscape, Environment, Agriculture and Food—Research Center, Instituto Superior de Agronomia, Associated Laboratory TERRA, University of Lisbon, Lisbon, Portugal

⁵CECAV - Animal and Veterinary Research Center, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

* mjoaofraqueza@fmv.ulisboa.pt

Excessive sodium intake is linked to several health risks, particularly cardiovascular diseases. In response, the World Health Organization (WHO) aims to reduce global salt consumption by 30% by 2030 [1], driving demand for lower-sodium food products. However, sodium chloride (NaCl) is essential for the safety and sensory quality of cured meat sausages (CMS) [2]. This study assessed the potential of potassium chloride (KCl) and encapsulated aromatic oleoresins (EO) as NaCl substitutes in CMS, evaluating their impact on product quality and shelf life.

Four CMS formulations were developed: C1 (1.5%NaCl), F1 (1%NaCl+0.5%KCl), F2 (1%NaCl+0.5%EO with 0.3%KCl), and F3 (1%NaCl+0.5%EO). The sausages underwent a curing process involving cold smoking and ripening over eight days followed by vacuum packaging and storage at 4°C. Physicochemical, microbial, and sensory analyses were conducted at multiple stages, including pre-stuffing, final product evaluation, and up to six months of storage. Results showed that water activity and pH remained unchanged across formulations. Colour analysis revealed that F2 CMS presented the highest a^* value (15.79), indicating enhanced reddish coloration. TBARS analysis showed significantly lower lipid oxidation in formulations containing KCl and oleoresins compared to the control. Microbiological analysis confirmed the absence of *Listeria monocytogenes* in 25 g of samples, with no significant differences in counts of *Enterobacteriaceae*, Lactic Acid Bacteria, or coagulase-negative Staphylococci. Sensory analysis with 97 consumers found no significant association between KCl inclusion and metallic taste perception. The JAR test indicated that 55% of consumers considered the control's saltiness ideal, while F1 (54%) met market readiness criteria [3]. However, F2 and F3 received slightly lower ideal salinity ratings (42% and 45%, respectively), suggesting the need for optimization of encapsulated oleoresin levels. In conclusion, replacing 33% of NaCl with KCl preserves the safety, quality, and sensory acceptance of CMS, presenting a promising strategy for sodium reduction in meat products.

Acknowledgements: The first author's scholarship was funded by the Portuguese Foundation for Science and Technology (FCT)—UI/BD/152824/2022. This research was funded by FCT under projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS), with financial support of the project AL4A-PROJ-LT1.3—Safety and acceptability assessment of green-label cured meat products.

References:

- [1] [WHO] World Health Organization, WHO global report on sodium intake reduction (2023) Geneva.
- [2] M.J. Fraqueza, M. Laranjo, M. Elias, L. Patarata, Curr. Opin. Food Sci., 40 (2021) 32–39.
- [3] L. Patarata, F. Carvalho, M.J. Fraqueza, Foods, 11 (2022) 796.

Enhancing catfish (*Clarias gariepinus*) fillet quality through optimized salting and smoking techniques

Busenur Özkan^{1,2} †, Marta Dias^{3†}, Maria José Fernandes^{1,2}, Maria Helena Fernandes^{1,2}, Patricia Bernardo^{1,2}, Maria Leonor Nunes³, Carla Pires^{3,4}, António Marques⁴, Juliana Pais-Costa³, Amparo Gonçalves⁴, Maria João Fraqueza^{1,2*}

¹ Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

²AL4AnimalS - Associate Laboratory for Animal and Veterinary Sciences

³ CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal

⁴ Division of Aquaculture, Upgrading and Bioprospection (DivAV), Portuguese Institute for the Sea and Atmosphere (IPMA IP), Portugal

†The two authors contributed equally to the article

* mjoaofraqueza@fmv.ulisboa.pt

Aquaculture is crucial for future food security at global, regional, and community levels, offering a stable protein source. Among farmed species, carp, tilapia, catfish, salmon, and shrimps are some of the most important that will contribute substantially to global food supply and security in the coming years, particularly in African and Asian countries. [1]. The preservation and processing of fish through pickling, drying, and smoking are traditional techniques that significantly affect their quality, safety, and sensory properties [2].

This study aims to develop a smoked prototype by using catfish *Clarias gariepinus* (increasingly prominent in global aquaculture) and evaluate the effects of different formulations and curing/smoking processing on chemical, microbiological, and sensory properties of the smoked product. Thus, four formulations were tested (different concentrations of NaCl, sodium lactate, and sugar) and three independent trials were carried out to identify the ideal formulation to obtain stable products from a physical, microbiological, and sensorial appealing point of view.

Fillets, prepared as business as usual, were hot smoked, and after a resting of 24h were vacuum packed, and stored at 4 °C. Selected analyses included physical, chemical, and microbiological parameters (pH, water activity, chlorides, colour, TBA, total aerobic, *Enterobacteriaceae*, lactic acid bacteria, and *Listeria* spp. counts), and sensory attributes (texture, flavour, and overall acceptability). The results obtained indicate good acceptance of all products (namely no *Enterobacteriaceae* or *Listeria* counts) and no significant differences among formulations. Now a second phase is following aiming to evaluate the shelf life associated with each formulation to choose the most appropriate one. These findings provide valuable insights into the aquaculture food industry, promoting the development of high-quality processed fish products.

This work aligns with EFSA's priorities and demonstrates how innovation addresses real-world challenges in the agri-food sector, fostering competitiveness through improved product quality and safety.

Acknowledgments: This work was developed under the frame of the Project Grant Agreement No. 101136739 INNOECOFOOD, financed by Horizon Europe and UK Research and Innovation, and it comprises an experienced multi-actor consortium of 20 partners in Europe and Africa and rural communities. We gratefully acknowledge FCT's support through the Programme Portugal2020 for CIIMAR (UIDB/Multi/04423/2021 and UIDB/Multi/04423/2022) and for the projects UIDB/00276/2020 (CIISA) and LA/P/0059/2020 (AL4AnimalS).

References:

- [1] K. Obeiero, P. Melenbroek, S. Drexler, A. Dagne, P. Akoll, R. Odong, B. Kaunda- Arara, H. Waidbacher, The Contribution of Fish to Food and Nutrition Security in Eastern Africa: Emerging Trends and Future Outlooks, Sustainability, 11 (2019) 1636.
- [2] S. Samples, The effects of processing technologies and preparation on the final quality of fish products, Trends in Food Science and Technology, 44 (2015), 131- 146.

***Listeria monocytogenes*: Dead or Just Pretending? Unveiling the VBNC State**

Beatriz Ferreira^{1,2}, Ana Rita Barata^{2,5,6}, M. Beatriz P. P. Oliveira^{1,3}, Gonalo Almeida^{2,4}

¹Faculdade de Farmácia da Universidade do Porto, Porto, Portugal

²Instituto Nacional de Investigação Agrária e Veterinária, Vila do Conde, Portugal

³REQUIMTE/LAQV, Dept. Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal

⁴Centre for Study in Animal Science (CECA), ICETA, University of Porto, 4050-083 Porto, Portugal

⁵Center for Research and Agro-environmental and Biological Technologies (CITAB) and Inov4Agro, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

⁶Center for Animal and Veterinary Science (CECAV), Associated Laboratory of Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

*goncalo.almeida@iniav.pt

Introduction: *Listeria monocytogenes* is a significant foodborne pathogen capable of entering a viable but non-culturable (VBNC) state when exposed to environmental stressors. In this state, cells retain metabolic activity but cannot be detected using standard culturing techniques, posing a critical challenge for food safety and public health. The presence of VBNC *L. monocytogenes* in food-processing environments raises concerns about the efficacy of conventional detection and control strategies [1,2].

Objective: This study aims to provide a systematic review of the mechanisms driving the induction, detection, and resuscitation of *L. monocytogenes* in the VBNC state. Through a comprehensive analysis of current literature and regulatory frameworks, we seek to highlight gaps in food safety protocols and propose improvements in pathogen monitoring.

Methods: A systematic search was conducted using the PubMed database, as well as European and North American regulatory guidelines. Duplicate records were removed, and relevant studies were selected based on predefined inclusion criteria, ensuring a comprehensive and unbiased review of the existing knowledge on *L. monocytogenes* in the VBNC state [3].

Results: The findings highlight the necessity for robust detection strategies to mitigate risks in food safety management. Current culture-based techniques underestimate the presence of VBNC *L. monocytogenes*, emphasizing the need for molecular detection methods such as qPCR and viability-based assays [4,5]. Additionally, factors such as biocide exposure, temperature fluctuations, and nutrient deprivation were identified as key stressors inducing the VBNC state. Resuscitation potential remains a critical concern, as certain conditions may allow *L. monocytogenes* to regain virulence and pose a renewed threat to public health [6].

Conclusion: Addressing the challenges posed by VBNC *L. monocytogenes* requires the integration of advanced detection methodologies and regulatory adaptations. Future research should focus on developing standardized protocols for VBNC detection and investigating the potential resuscitation mechanisms of this pathogen. Strengthening food safety policies with these insights will be crucial in minimizing the risks associated with VBNC cells in food production and public health [7,8].

Acknowledgments: GenoPhenoTraits4Persistence - Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment, reference (PTDC/BAA-AGR/4194/2021), financed by national funds from FCT, I.P

References:

- [1] X. Zhao, Z. Wei, C. Lin, *Front. Microbiol.*, 8 (2017) 123.
- [2] K. Dong, P. Yang, D. Rao, *Compr. Rev. Food Sci. Food Saf.*, 19 (2020) 149.
- [3] T. Brauge, C. Leleu, G. Midelet, *Food Microbiol.*, 92 (2020) 103.
- [4] N. Wideman, J.D. Oliver, P.G. Crandall, *Microorganisms*, 9 (2021) 456.
- [5] A. Lotoux, E. Maillard, H. Bierre, *Front. Cell. Infect. Microbiol.*, 12 (2022) 112.
- [6] B. Liu, X. Zhang, N. Zhang, *Microbiol. Res.*, 250 (2023) 210.
- [7] V. Besnard, M. Federighi, J.M. Cappelier, *Food Microbiol.*, 17 (2000) 159.
- [8] J.M. Cappelier, V. Besnard, P. Velge, *EDP Sciences*, 36 (2005) 97.

Impact of cognitive style on the evaluation of consumer perception of different orange juices: a case study with projective mapping approach

Bruna Barbosa^{1,2}, Diego Martínez^{1,3}, Célia Rocha^{1,2}, Maria João Monteiro³, Rui Costa Lima¹, Maria João Alegria⁴, Luís Miguel Cunha^{2*}

¹Sense Test, Vila Nova de Gaia, Portugal

² GreenUPorto/Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Porto, Portugal

³ Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

⁴ Sumol+Compal, Almeirim, Portugal

*lmcunha@fc.up.pt

Projective Mapping (PM) is a fast technique for product comparison [1], where the participants place the samples on a map, grouping the ones perceived as similar. It often incorporates descriptive profiling methods, such as Ultra Flash Profiling [2]. Cognitive styles affect the multiple tactics people use to process information [3] and should be considered when selecting a consumer panel. This study evaluated consumer perception of different orange nectar products while segmenting responses based on participants' cognitive styles.

One hundred and fifty participants assessed seven orange juice samples over two separate sessions. In the first session, participants evaluated overall liking using a 9-point hedonic scale. Subsequently, a projective mapping technique was employed, where participants positioned the samples on a screen-based map, based on perceived similarity/dissimilarity. After positioning the samples, they were invited to describe them using the Ultra Flash Profiling approach. In the second session, the projective mapping technique was repeated using only the packaging of the products. At the end of session two, participants completed the Cognitive Style Index questionnaire, segmenting the participants into five different styles ('intuitive', 'quasi-intuitive', 'adaptive', 'quasi-analytic' and 'analytic') [4].

The results revealed significant differences in the overall liking scores. The projective mapping analysis reveals differences in the perception of orange juice. First, participants with an RV coefficient below 0.450 were removed once their contribution was too low for the final consensus perception map. Samples with a higher overall liking score are related to pleasant attributes, with pulp and thick texture and balanced and intense taste. The cognitive style segmentation brings a higher sample discrimination with the 'intuitive' participants characterising products in greater detail, on the tasting samples evaluation. When evaluating packaging, these differences in product discrimination according to cognitive style are less marked.

Acknowledgements: This work has received funding from "Project cLabel+ POCI-01-0247-FEDER-046080", funded by COMPETE2020, Portugal2020, Lisboa2020 and FEDER. Author Bruna Barbosa acknowledges Non-Academic Doctoral grant No. 2023.03467.BDANA, funded by the Foundation for Science and Technology (FCT) and supported by Sense Test, Lda. Authors Barbosa, Rocha and Cunha acknowledge additional support from FCT through UID 05748: GreenUPorto - *Centro de Investigação em Produção Agroalimentar Sustentável*.

References:

- [1] M. Jervis., M. Drake, *Journal of Sensory Studies*, 29 (2014) 242-243.
- [2] R. Moss, M.B. McSweeney, *Journal of Sensory Studies* 2 (2022).
- [3] *Cognitive Styles and Learning Strategies. Understanding Style Differences in Learning and Behaviour* Richard Riding, Stephen Rayner, (1st Edition), London, David Fulton Publishers, (1998).
- [4] Allinson, C.; Hayes, J. *Journal of Management Studies*, 33 (1), (1996).

Effect of storage time and temperature on the acceptability of canned mackerel with vegetables

Ana Boticas^{1,2}, Luís Baião^{1,2}, Ana Cação³, Helga Augusto³, Célia Rocha^{1,2*}, Rui Costa Lima¹, Luís Miguel Cunha^{2*}

¹ Sense Test, Vila Nova de Gaia, Portugal

² GreenUPorto/Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Porto, Portugal

³ Cofisa, Figueira da Foz, Portugal

* Imcunha@fc.up.pt

In canned foods, storage time and temperature can influence product quality, modifying sensory properties such as texture, flavour, and colour [1]. This study aimed to assess the impact of storage time and temperature on the acceptability of canned mackerel with vegetables in tomato sauce by comparing different samples stored under different conditions of time and temperature. The samples were evaluated three times: immediately after stability and sterility, 5 months after, and 8 months after. This evaluation was performed for sample storage at room temperature and 40° C (only for T5 and T8). In each test, an independent panel of 100 consumers evaluated the samples on overall liking and sensory attributes, following the ISO standard 11136 and internal procedure PR13, an accredited method of Sense Test, in which consumers were asked to evaluate the overall liking using a 9-point hedonic scale and afterwards were also asked to make an open comment about the different aspects that they freely found on the product. The comments were then coded into positive and negative aspects.

The results show that all the samples had high average scores for overall liking (>7.5). When comparing the products at room temperature (T0 and T5), there were no significant differences in the overall liking. As for the results obtained at the same storage time, T5, and at different storage temperatures, room temperature and 40°C, there were no significant differences in overall liking.

This study was important for understanding the eventual impact of storage time and temperature on the overall quality and liking of tinned foods, specifically tinned mackerel with vegetables.

Acknowledgements: This work has received funding from “*Agenda Mobilizadora Pacto da BioEconomia Azul*”, funded by the Resilience and Recuperation Program in Portugal, financed by the Next Generation EU funds. Authors Baião, Rocha and Cunha acknowledge additional support from FCT through UID 05748: GreenUPorto - *Centro de Investigação em Produção Agroalimentar Sustentável*.

References:

- [1] Šopík, T., Lazárková, Z., Salek, R. N., Talár, J., Purevdorj, K., Buňková, L., Foltin, P., Jančová, P., Novotný, M., Gál, R., & Buňka, F. (2022). . Foods, 11(14), (2004). <https://doi.org/10.3390/foods11142004X>.

Sensory evaluation and consumer liking of bread enriched with *Tenebrio Molitor* flour

Bruna Barbosa^{1,2,3*}, Daniela Campelo^{1,3}, Vera Fernandes⁴, Joana Pereira⁴, António Gil⁴, José Cordeiro⁴, Rui Costa Lima¹, Célia Rocha^{1,2*}

¹ Sense Test, Lda, Rua Zeferino Costa, 341, 4400-345 Vila Nova de Gaia

² GreenUPorto - Sustainable Agrifood Production Research Centre / Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Campus de Vairão, Rua da Agrária 747, 4485-646 Vila do Conde

³ Faculty of Nutrition and Food Science of the University of Porto, Porto, Portugal

⁴ Auchan Retail Portugal, Estrada de Paço de Arcos 48 A, 2770-129 Paço de Arcos

* celia.rocha@sensetest.pt

Edible insects are a sustainable, nutrient-rich alternative to meat, requiring fewer resources and producing lower emissions. Rich in essential amino acids, vitamins, and minerals, their protein offers a viable solution to rising global protein demand while reducing environmental impact [1,2]. This research assessed the effect of incorporating *Tenebrio molitor* flour on three bread types — brioche, cereal, and wheat— each with incorporation levels of 0% (control), 5% and 10%.

Three independent panels of 65 individuals willing to taste insect-based products evaluated the three samples of bread within each type. Participants were invited to rate overall liking, and their appreciation of appearance, odour, texture, and taste using a 9-point hedonic scale. Additionally, they indicated their purchase intention (yes/no) and provided an open comment, reflecting on the positive and negative aspects of the samples.

Results showed that the liking scores were high for all the samples. However, an increase in the incorporation of *Tenebrio molitor* flour (10%) led to a decrease in overall liking. In brioche bread, significant differences were found in appearance, odour, and taste, with a marked decrease in liking at the highest incorporation level. Cereal and wheat bread maintained similar overall liking, but flavour evaluations showed a significant decrease at 10% incorporation.

Qualitative analysis of the open comments indicated that darker colours and denser textures were less appreciated in the enriched samples. Negative perceptions of taste, particularly an unfamiliar or undesirable taste, were more pronounced in brioche at 10% incorporation. The purchase intention was generally lower for the 10% incorporation samples, but a 5% incorporation slightly increased purchase intention in cereal and wheat bread.

Tenebrio molitor flour can be integrated into bread formulations, though the incorporation at higher concentrations may negatively impact liking. The findings highlight the need for further formulation adjustments to enhance sensory appeal.

Acknowledgements: This work has received funding from “Agenda Mobilizadora InsectERA – um novo setor bioindustrial”, funded by the Resilience and Recuperation Program in Portugal, financed by the Next generation EU funds. Author Barbosa acknowledges Non-Academic Doctoral grant No. 2023.03467.BDANA, funded by the Foundation for Science and Technology (FCT) and supported by Sense Test, Lda. Authors Barbosa and Rocha acknowledge additional support from FCT through UID 05748: GreenUPorto - Centro de Investigação em Produção Agroalimentar Sustentável.

References:

- [2] L.M. Cunha, J.C. Ribeiro, in *Edible Insects in the Food Sector: Methods, Current Applications and Perspectives* (pp. 57-71). G. Sogari, C. Mora, D. Menozzi (Eds.), Springer International, (2019).
- [3] B. Puteri, M. Oehlmann, B. Jahnke, *Food Research International*, 196 (2024) 114994.

The influence of typefaces on the emotional response and sensory evaluation of food products

P.M. Sousa¹, L. Chen², H. Seo², P. García-Segovia³, L.M. Cunha^{1*}

¹ GreenUPorto/Inov4Agro, DGAOT, Faculdade de Ciências, Universidade do Porto, Porto, Portugal;

² Department of Food Science, University of Arkansas, Fayetteville, AR 72704, USA;

³ Dpto Tecnología de Alimentos, Universitat Politècnica de València, Valencia, Spain;

* Imcunha@fc.up.pt

Fonts, also known as typefaces, can play an important role in shaping consumers' emotions, perceptions and experiences [1]. Different typefaces can evoke various feelings, reinforcing or altering the conveyed message. Thus, typeface selection becomes particularly important in sensory testing, where responses can be subtly influenced by how information is presented.

This work aimed to assess consumers' emotional response to different fonts and explore the influence of fonts on the overall liking, aroma intensity and willingness to purchase commercial tomato juice.

Ninety-two participants were recruited, students and staff from the University of Arkansas, USA. In the first phase, participants were instructed to match the 12 emotional word pairs of the circumflex-inspired Valence × Arousal [2] emotion questionnaire with 20 different fonts, according to their perception, leading to the selection of the four fonts with the highest scores in each of the four emotional dimensions (Active, Happy, Passive and Unhappy). In a second phase, sensory evaluation was conducted to assess the influence of the selected fonts on the assessment of the evoked emotions, overall liking, aroma intensity and willingness to purchase tomato juice.

The four typefaces with the highest emotional impact on the Active, Happy, Passive and Unhappy dimensions were Verdana, Candara, Consolas and Gigi.

The sensory tasting results show no significant impact ($p > 0.05$) of the typefaces on overall liking, taste intensity or purchase intention of tomato juice. Nevertheless, Verdana and Candara were strongly associated with active and happy emotions, while Gigi was more closely related to passive and unhappy feelings. Consolas has a stronger association with passive and neutral states.

Typefaces can shape emotional perceptions without necessarily affecting the direct sensory evaluation of the product, meaning that additional research is needed to investigate their impact over repeated exposure.

Acknowledgements: Author Sousa is grateful for the PhD scholarship nº SFRHUI/BD/152303/2021, funded by the FCT, Portugal and the Fulbright scholarship, funded by the Fulbright Portugal programme. Authors Sousa and Cunha acknowledge support from the strategic project UID 05748: GreenUPorto - *Centro de investigação em Produção Agroalimentar Sustentável*, funded by national funds via FCT (Foundation for Science and Technology - Portuguese Ministry of Science, Technology and Higher Education).

References:

- [1] A. Shaikh, B. Chaparro, D. Fox. Perception of fonts: Perceived personality traits and uses. *Usability news*, 8(1), 1-6 (2006)
- [2] S. Jaeger, C. Roigard, S. Chheang. The valence× arousal circumplex-inspired emotion questionnaire (CEQ): Effect of response format and question layout. *Food Quality and Preference*, 90 (2021).

Effect of mixing alternative proteins on the techno-functional properties of the resulting mixtures

Ricardo Isaías^{1,2,3}, Luís Loureiro^{1,4}, Rui Rodrigues^{1,4}, Diana Vilas Boas^{1,4}, José C. Ribeiro², Célia Rocha^{2,3}, Luís M. Cunha², António A. Vicente^{1,4,*}

¹ CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal

² GreenUPorto/Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Vairão, Portugal

³ Sense Test, Lda., Vila Nova de Gaia, Portugal

⁴ LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

* avicente@deb.uminho.pt

Alternative proteins are a promising market in the food sector since they provide sustainable solutions to the currently consumed proteins. However, poor sensory or techno-functional properties constitute hindrances to the success of these protein products [1,2]. Therefore, the objectives of this work were to map the main techno-functional properties of two alternative protein sources (i.e., lupin beans and edible house crickets) and improve them by mixing those proteins in different ratios.

Lupin protein concentrate (L) and cricket (*Acheta domesticus*) flours (whole -WC- and defatted -DC-) were used. All samples were tested for main technological parameters (e.g., solubility) using standard methods. Afterwards, samples were mixed (insect/pulse) in 1:1, 1:3, and 1:9 ratios, and their solubility, foaming capacity and stability, emulsifying and gelling capacities, and water/oil absorption capacities (WAC/OAC) were measured. Structural characterisation was performed (DLS, Particle Size Distribution, SEM) to understand how the several proteins interact with each other.

There was no gel/emulsion formation, while solubility showed a similar pattern: a drastic reduction in mildly acidic environments. Nevertheless, WC/DC solubility increased significantly when mixed, not affecting this property of L majorly (until 50% of WC was added). Foaming exhibited a similar trend, especially when DC was used, though no relevant foaming stabilities were revealed. There was a significant increase in WAC with insect addition. DLS seemed to indicate protein structural stability even when mixed (lower zeta potential), and overall, there was an increase in particle size with the increasing incorporation of crickets. SEM showed particles with different shapes and sizes, some smoother/more porous (L) and some irregular/rougher (WC).

There was a clear interaction between WC/DC and L, which appeared synergistically, with improvements being detected on several functional properties. Various treatments can be applied to further increase functionality, and thereby formulate food products with these novel, alternative and more sustainable ingredients.

Acknowledgements: Author Isaías acknowledges Non-Academic Doctoral Grant No. 2022.12384.BDANA, funded by the Portuguese Foundation for Science and Technology (FCT). Authors Isaías, Loureiro, Rodrigues, Vilas Boas and Vicente acknowledge support from the FCT under the scope of fundings UIDB/04469/2020 (CEB), LA/P/0029/2020 (LABBELS). Authors Isaías, Ribeiro, Rocha and Cunha declare additional from the strategic project UID 05748: GreenUPorto - *Centro de investigação em Produção Agroalimentar Sustentável*, funded by national funds via FCT.

References:

- [1] D. Hagmann, M. Siegrist, C. Hartmann, Public Health Nutrition, 22 (2019) 2448-2459.
- [2] L. Grossmann, J. Weiss, Annual Review of Food Science and Technology, 12 (2021) 93-117.

Development of functional insect-based food products: insights from a market study

Valquíria Francisco¹, José Carlos Ribeiro¹, Luís Miguel Cunha^{1*}

¹ GreenUPorto - Sustainable Agrifood Production Research Centre / Inov4Agro, DGAOT, Faculty of Sciences, University of Porto, Campus de Vairão, Rua da Agrária 747, 4485-646 Vila do Conde, Portugal

*lmcunha@fc.up.pt

Consumers with heightened interest in food products' health and nutritional benefits generally present higher willingness to try insect-based food products. As such, the development of functional products, such as protein bars or protein concentrates/isolates, is a promising strategy to increase consumer acceptance.

The present study is market research on protein bars and protein concentrates/isolates sold in Portuguese retail stores, to identify key characteristics for the development of similar insect-based food products. For each product, information was retrieved pertaining product name, brand, price, ingredients list, nutritional characteristics, and nutritional claims present on the package.

For protein bars (n = 109), protein extracts (e.g., concentrate, isolate, hydrolysate) from milk (61.5%) and soy (48.6%) were the most used protein ingredients. Price was heavily influenced by the sugar content, with the most expensive bars being associated with lower sugar content. On the other hand, the protein content or *Protein Claim* level (% of the energy value of the food provided by protein) were not directly correlated with the bars' price. However, most of the products still presented high protein content (around 25.0%-31.0%) and *Protein Claim* levels (22.0%-34.0%). Of the claims present on the package, those related to protein were the most frequent (41.0%), with most protein-related claims being present as the % of protein of the products (50.9%).

For protein concentrates/isolates (n = 319), the most common protein sources were derived from whey: concentrate (51.7%), isolate (43.9%) and hydrolysate (25.1%) while plant-based sources (e.g. pea, soy, rice) had much lower relative frequencies. Price was significantly influenced by nutritional characteristics, with the most expensive products presenting the highest *Protein Claim* (88.0%) and protein (81.2%) levels, and the lowest energy (369 kcal/100 g), lipid (2.33 g/100 g), saturated fat (1.2 g/100 g), carbohydrates (5.7 g/100 g), and sugars (2.0 g/100 g) content.

Acknowledgments: Author J. C. Ribeiro acknowledges the PhD Researcher Contract within the "VIIAFOOD" Agenda, approved in the PRR programme (Notice No. 02/C05-i01/2022) under No. C644929456-00000040, for the RTD project "HIT-C—High Integration Technology for Consumer Insights. Authors, J.C. Ribeiro, and L.M. Cunha acknowledge additional support from FCT through UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável.

‘Maçã de Alcobaça’ PGI: determinants of choice by Portuguese consumers

Bruna Silva¹, Ana Pinto de Moura¹, and Luís Miguel Cunha^{2,*}

¹ GreenUPorto /INOV4Agro, DCEt, Universidade Aberta, Porto, Portugal

² GreenUPorto /INOV4Agro, DGAOT, Faculty of Sciences, University of Porto, Vila do Conde, Portugal

* Imcunha@fc.up.pt

The *Maçã de Alcobaça*, protected geographical indication (PGI) refers to apples produced in Alcobaça, West region of Portugal, benefiting from its temperate climate and fertile soils. The fact that the fruits are certified under the PGI scheme, ensures authenticity and adherence to traditional farming methods [1]. A questionnaire was created and implemented using the Lime Survey platform to evaluate the main determinants of choice of these *Maçã de Alcobaça* PGI apples among Portuguese consumers, following an adaption of the Food Choice Questionnaire (FCQ) [2], as suggested by Cunha et al. (2018) [3] and assess the consumption pattern of this product within the context of regular dietary habits. To ensure greater representativeness, sampling criteria were implemented based on participants' gender, age group, education, and geographic location. FCQ data was reduced following factorial analysis and dimensions scores were computed. Participants were clustered, following a k-means algorithm based on their FCQ dimensions scores. Out of 275 participating adults from both genders residing in Portugal, only 166 were validated. The results showed that the main criteria for choosing the *Maçã de Alcobaça* IGP identified were "sensory appeal", "natural content", "health" and "concern with origin", and it was also found that women value more factors such as "health", "price" and "convenience", while men are more sensitive to the "sensory appeal" of the product. Participants were clustered into "Natural", "Engaged" and "Practical", with the "Engaged" consuming more *Maçã de Alcobaça* PGI apples per week (app. 2.5), preferably of the 'Fuji' variety, and with a distinct consumption of less popular varieties, such as 'Casanova de Alcobaça' or 'Red Delicious', with a clear preference for eating raw whole apples with skin, and strongly valuing its food safety attributes. This work sets up new perspectives on the promotion and valuation of more *Maçã de Alcobaça* PGI apples.

Acknowledgements: Authors Silva, Moura, and Cunha acknowledge support from the strategic project UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável, funded by national funds via FCT (Foundation for Science and Technology - Portuguese Ministry of Science, Technology and Higher Education).

References:

- [1] Tibério, M. (2004). Construção da qualidade e valorização dos produtos agro-alimentares tradicionais - Estudo da Região de Trás-os-Montes. *Revista Portuguesa de Estudos Regionais*, 17, 7–24.
- [2] Steptoe, A., Pollard, T. M., & Wardle, J. (1995). Development of a measure of the motives underlying the selection of food: the food choice questionnaire. *Appetite*, 25(3), 267-284.
- [3] Cunha, L. M., Cabral, D., Moura, A. P., & de Almeida, M. D. V. (2018). Application of the Food Choice Questionnaire across cultures: Systematic review of cross-cultural and single country studies. *Food Quality and Preference*, 64, 21-36.

Innovation in the technological production of aged honey spirit with acacia wood

Carlos Antunes¹, Soraia I. Pedro², Ilda Caldeira^{3,4}, Ofélia Anjos^{2,5*}

¹ AProSer – Associação de produtores do Conselho da Sertã. 6100-601 Sertã, Portugal
carlosalbertoantunescb@gmail.com

² CERNAS-IPCB - Research Centre for Natural Resources, Environment and Society,
Polytechnic University of Castelo Branco, 6000-084 Castelo Branco, Portugal.
soraia.pedro@ipcb.pt;

³ INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Polo de Inovação de Dois
Portos, Quinta de Almoinha, 2565-191 Dois Portos, Portugal. ilda.caldeira@iniav.pt

⁴ MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE –
Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada,
Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

⁵ CBP-BI, Chromatography and Spectroscopic laboratory, 6000-084 Castelo Branco, Portugal
*ofelia@ipcb.pt

The maturation of spirit drinks with wood is a key process that affects its quality by enriching its aroma, sensory properties, and colour attributes. Honey spirit, obtained through honey fermentation and distillation, has a distinctive aromatic profile that differentiates it from traditional spirits drinks.

This study evaluated an innovative approach to ageing honey spirit with different Acacia wood species to assess their impact on its volatile and colour characteristics. The three months ageing process was conducted on a laboratory scale using medium-toasted wood from three Acacia species. The honey spirit samples were analysed for volatile compounds using gas chromatography (GC-FID), colour attributes and Fourier transform Raman spectroscopy (FT-Raman).

The alcohol content of the samples remained stable, ranging from 55.9% to 56.5%, with significant colour variations observed. Among the major volatile compounds, methanol levels were minimal, while isoamyl alcohols and isobutanol were the most prevalent. The honey spirit aged with Acacia wood displayed distinct colour differences, with varying intensities of golden hues, indicating the influence of the wood species on the final product.

The Raman technique successfully differentiated between the analysed honey spirit samples, revealing compositional and ageing-related variations associated with the type of Acacia wood used. These findings highlight the potential of Acacia wood in honey spirit, offering new possibilities for product innovation and differentiation in the spirits market.

Acknowledgments: This work was supported through the following projects: FCT/MCTES (PIDDAC), UIDB/00681/2020 [CERNAS-IPCB] and UIDB/05183/2020 [MED&CHANGE].



Co-Processed olive oils with culinary herbs: a new food preparation

Beatriz Azinheira¹, Cecilia Gouveia¹, Ofélia Anjos^{1,2,3}, Suzana Ferreira-Dias^{4,5} & Fátima Peres^{1,4}

¹IPCB - Polytechnic Institute of Castelo Branco, 6000-909 Castelo Branco, Portugal; fperes@ipcb.pt

²CERNAS-IPCB - Research Centre for Natural Resources, Environment and Society, Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal; ofelia@ipcb.pt

³CBP-BI - Chromatography and Spectroscopic laboratory, 6000-084 Castelo Branco, Portugal

⁴LEAF - Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal,

⁵Laboratório de Estudos Técnicos, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal; suzanafdias@isa.ulisboa.pt

* bazinheira@ipcbcampus.pt

Flavouring virgin olive oil (VOO) with different ingredients is an increasing trend to obtain new products. This type of food preparation is highly appreciated especially by consumers outside the Mediterranean countries. An innovative method of enrichment VOO is through co-processing, also known as co-extraction, based on the addition of culinary herbs, during the olive crushing or in the malaxation step. The co-processing method can improve the sensory and nutritional properties, as well as the shelf life of the final food preparations.

The present work presents studies, performed for the development of new products through the co-extraction of ripe olives of cv. 'Cornicabra', with low fruity flavour intensity and subject to frost, in presence of juniper berries (*Juniper communis* L.), oregano (*Origanum vulgare* L.), bay leaf (*Laurus nobilis* L.) or breckland thyme (*Thymus serpyllum* L.). The addition of culinary herbs was carried out during the extraction process in malaxation. Quality criteria (acidity, peroxide value, UV absorbance, and organoleptic evaluation) and the main fatty acids were evaluated. Fourier transform near infrared spectroscopy (FT-NIR) was used to study the variability of the co-processed products. The addition of juniper berries showed the most significant changes in olive oils, with an increase in oleic and linoleic acids and in K₂₇₀ and K₂₃₂ values. Moreover, the co-processing of olives with bay leaf showed the lowest changes in chemical composition. In addition, all the new products obtained by co-extraction showed improved sensory notes responsible for an increased value-added of these products.

FT-NIR spectroscopy demonstrated high effectiveness in the characterization and identification of different co-processed oils, offering a reliable analytical tool for this purpose.

Acknowledgments: This study was developed in the framework of the project PD24-00014 OLIVOTEC - "Valorização Tecnológica de Azeites de Territórios do Interior de Portugal", Programa Promove da Fundação "la Caixa", funded by Fundação La Caixa and FCT – Fundação para a Ciência e a Tecnologia, Portugal.. The grant received by Beatriz Azinheira is also supported by OLIVOTEC.

The impact of ingredient concentration on the stability of basil essential oil nanoemulsions

Andrei Pavei Battisti¹, Jéssica de Matos Fonseca¹, German Ayala Valencia¹, Alcilene Rodrigues Monteiro^{1,*}, Ana Isabel Bourbon², Lorenzo Pastrana Castro²

¹ Laboratory of physical properties of foods, Chemical and Food Engineering Department, Federal University of Santa Catarina, UFSC, 88040900, Florianopolis, SC, Brazil

² International Iberic Nanotechnology Laboratory, INL, 4715-330, Braga, Portugal

* alcilene.fritz@ufsc.br

This study explores the impact of processing variables on the stability of nanoemulsions containing basil (*Ocimum basilicum* L.) essential oil and sunflower oil, stabilized by saponins (a natural emulsifier). A completely randomized experimental design was applied to identify the optimal formulation for long-term stability.

A total of sixteen experiments were performed using high energy to obtain nanoemulsions with several concentration ratios of saponins, essential oil, and sunflower oil. The results demonstrated that formulations with a surfactant-to-oil ratio (SOR) of 1:1 or higher and an essential oil-to-sunflower oil (EO/SFO) ratio of 3:7 exhibited superior stability for at least 120 days. These findings highlight the potential of saponin-stabilized nanoemulsions for enhancing the bioactivity and controlled release of natural compounds, offering a promising approach for sustainable food protection and biodegradable packaging solutions.

Acknowledgments: The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, for financial support. The authors are grateful to CAPES-PRINT, Project 88881.310728/2018-01. Ana Isabel Bourbon acknowledges funding by FCT, through the individual scientific employment program contract (2020.03447.CEECIND).

References:

- [1] J.M. Beltrame, R.A. Angnes, L.U.R. Chiavelli, W.F. da Costa, S.F. Montanher, M.F. da Rosa, V. da S. Lobo, A.M. Pomini, *Journal of Essential Oil Bearing Plants*, 17 (2014) 658–663.
- [2] L.T. Cardoso, B. Alexandre, F.A. Cacciatore, Y.V. da S. Magedans, A.G. Fett-Neto, R.V. Contri, P. da S. Malheiros, *Food Research International* 168 (2023).
- [3] S. da Silva Gündel, M.C. Velho, M.K. Diefenthaler, F.R. Favarin, P.M. Copetti, A. de Oliveira Fogaça, B. Klein, R. Wagner, A. Gündel, M.R. Sagrillo, A.F. Ourique, *J Drug Deliv Sci Technol* 46 (2018) 378–383.
- [4] J. de M. Fonseca, T.C. Trevisol, G.A. Valencia, A.R. Monteiro, *Bio-Based Nanoemulsions for Agri-Food Applications* (2022) 387–398.
- [5] N. Riquelme, R.N. Zúñiga, C. Arancibia, *LWT* 111 (2019) 760–766.
- [6] D.J. McClements, C.E. Gumus, *Adv Colloid Interface Sci* 234 (2016).

Survival and proliferation of foodborne pathogens in mung bean sprouts irrigated with contaminated water

Joana Gomes*, Joana Barbosa & Paula Teixeira

Universidade Católica Portuguesa, CBQF—Centro de Biotecnologia e Química Fina—
Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal²

* jcgomes@ucp.pt

The rise of healthy lifestyle trends has contributed to a growing consumption of ready-to-eat products, including vegetables and sprouts. Pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia coli* have been linked to foodborne outbreaks resulting from contaminated produce.¹ Once in contact with plant tissues, these microorganisms can adhere to and persist on the plant for extended periods of time. The initial contamination level plays a crucial role in determining their survival, spread, and persistence within plant tissues.²

This study aimed to assess the potential exposure to *L. monocytogenes*, *Salmonella enterica*, and *E. coli* from contaminated irrigation water during sprout production.

Mung bean irrigation water was inoculated with a cocktail of *L. monocytogenes* (two serotypes), *E. coli*, and *S. enterica* (four serotypes) at a final level of 6 log CFU/ml. Mung beans were germinated for 3 days in the dark at approximately 25 °C. Pathogen survival was evaluated through plate count enumeration. After 3 days, pathogen counts increased by around 2 log CFU/g for *L. monocytogenes*, 4 log CFU/g for *E. coli*, and 3 log CFU/g for *S. enterica*.

These preliminary results indicate that when irrigation water is contaminated with these pathogens, they can survive and proliferate in mung bean sprout tissues. This represents a significant public health concern, as sprouts are often consumed raw, providing optimal conditions for pathogen growth. Future research will focus on the impact of lower initial contamination levels and the behaviour of pathogens in other edible plant tissues.

Acknowledgments: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project GenoPhenoTraits4Persistence- Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment (PTDC/BAA-AGR/4194/2021). We would like to thank the scientific collaboration under the FCT project UIDB/50016/2020. Financial support for J. Gomes was provided by a doctoral fellowship 2021.06027.BD (FCT).

References:

- [1] G. A. Thomas, T. P. Gil, C. T. Müller, H. J. Rogers, C. N. Berger, *Food Microbiology*, 117 (2024) 104389.
- [2] O. O. Alegbeleye, I. Singleton, A. S. Sant'Ana, *Food Microbiology*, 73 (2018) 177-208.

Assessment of quality factors and shelf life of industrially processed fresh-cut pineapple

**Carolina Correia^{1,2}, Ana Cristina Ramos^{1,3}, Nelson Pereira^{1,2}, Cristina Roseiro^{1,3},
Helena Gonçalves¹, Margarida Moldão², Marta Abreu^{1,2*}**

¹INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Unidade de Tecnologia e Inovação, Oeiras, Portugal

²LEAF - Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, ULisboa, Lisboa, Portugal

³GeoBioTec - Geobiociências, Geoengenharias e Geotecnologias, FCT-UNL, Caparica, Portugal

**marta.abreu@iniav.pt*

Minimally processed pineapple (MP) is significant in the fresh-cut products market due to its appreciated sensory characteristics and antioxidant composition, particularly its high ascorbic acid content. However, its shelf life is short, often less than 15 days, and typically does not exceed one week. The main preservation challenges include juice release, changes in sensory traits (colour, shine, and firmness), development of off-flavours and odours, and excessive microbial growth.

This study evaluated the shelf life of minimally processed pineapple (Extra Sweet Gold variety), industrially produced and packed in two PET packaging formats (1 kg and 0.4 kg), identifying factors responsible for the loss of quality. The Extra Sweet Gold variety used in the processing met ripeness criteria ($\geq 12^\circ\text{Brix}$) and was slightly immature (titratable acidity of 0.8 g citric acid/100 g), with a high ascorbic acid content (76.9 ± 6.1 mg/100 g). The two PET packaging formats did not significantly ($p < 0.05$) influence the quality changes of MP pineapple stored for nine days at $5 \pm 1^\circ\text{C}$. Sensory evaluation showed marginal changes in attributes (colour, appearance, aroma, sweetness, acidity, firmness, and juiciness) compared to day 0, except for a pronounced off-flavour on day 7. Ascorbic acid and total phenolic content degraded by approximately 35% and 15%, respectively. Despite ascorbic acid loss by day 4, the remaining levels were significant ($\approx 30.1 \pm 3.3$ mg/100 g). Active atmosphere modification could extend shelf life, as passive modification did not meet the recommended gas composition (O_2 and CO_2). Excessive yeast growth, linked to unacceptable off-odours, compromised fresh quality from day 7 (≈ 6.0 Log10CFU/g), revealing the critical factor limiting shelf life.

Keywords: antioxidant compounds, ascorbic acid, modified atmosphere packaging, shelf life.

Exquisite cuisine: development of novel pudding and mousse recipes based on red macroalgae

Silvia Lomartire¹, João Cotas¹, L. Pereira¹, Ana M. M. Gonçalves^{1,2,*}

¹Marine Resources, Conservation and Technology, Marine Algae Lab, CFE—Centre for Functional Ecology: Science for People & Planet, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal;

²Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal;

**amgoncalves@uc.pt*

Macroalgae, consumed historically in eastern countries, has gathered western countries attention as an alternative and sustainable food source, demanded through consumer awareness regarding nutrition and at the same time environmentally friendly food production. Even though there is integration of macroalgae components in western diets and products, these either come as a utilization of specific compounds (such as agar and carrageenan) or associated to Asiatic cuisine (Nori consumption in Sushi), therefore not fully integrated as a daily ingredient in western diets [1,2].

In an effort to change the paradigm, and under the scope of the project MENU, a functional dessert was formulated based on whole macroalgae compounds, in order to better utilize the supported beneficial compounds within macroalgae organisms. Our research group developed novel recipes of pudding and mousse, being the essential ingredient the red macroalgae. Posteriorly to the product optimization, the group visited several schools in the Centre and Centre- South region of Portugal to conduct workshops regarding macroalgae ecology and the health benefits, with the degustation of the dessert by the students and schoolteachers for sensorial analysis. Inquiries were handed out to students between 15-20 years old, for them to perform sensorial evaluation of the product.

Overall, the product had satisfactory results and acceptance, still some students showed some resistance, which could infer about the dietary habits of the population in this age gap. Literacy regarding the marine environment and biotechnological potentialities needs further activities of dissemination in the different school grades, from primary to secondary grade, potentiating the acceptance and introduction of macroalgae food products in consumer's diet.

Acknowledgments: The authors acknowledge the Centre for Functional Ecology—Science for People and the Planet (CFE; UIDB/04004/2020; <https://doi.org/10.54499/UIDB/04004/2020>), financed by FCT/ MCTES through national funds (PIDDAC) and the Associate Laboratory TERRA (LA/P/0092/2020; <https://doi.org/10.54499/LA/P/0092/2020>). This research was financed by the project MENU—Marine Macroalgae: Alternative recipes for a daily nutritional diet (FA_05_2017_011), funded by the Blue Fund under Public Notice No. 5-Blue Biotechnology and co-financed by AlgaMar4antivirus—Marine Macroalgae in the prevention and treatment of ANTIViral diseases (Coronavirus SARS-CoV-2) (FA_07_2018_009), funded by the Blue Fund under Public Notice No. 7/2018—Scientific Research and Marine Technology.

References:

- [1] Lomartire, S., Gonçalves, A.M.M., Novel Technologies for Seaweed Polysaccharides Extraction and Their Use in Food with Therapeutically Applications—A Review. *Foods*, 11 (2022) 2654.
- [2] Pacheco, D., Araújo, G., Silva, J.W.A., Cotas, J., Gonçalves, A.M.M., Pereira, L., Red Seaweeds: Their Use in Formulation of Nutraceutical Food Products. In: Ranga Rao, A., Ravishankar, G.A. (eds) *Sustainable Global Resources of Seaweeds Volume 2*. Springer, Cham, (2022).

Characterization of the nutritional composition of four macroalgae species from the Western Portuguese coast to food diet alternatives

Silvia Lomartire¹, João Cotas¹, L. Pereira¹, Ana M. M. Gonçalves^{1,2,*}

¹Marine Resources, Conservation and Technology, Marine Algae Lab, CFE—Centre for Functional Ecology: Science for People & Planet, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal;

²Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal;

*amgoncalves@uc.pt

Due to increasing global population, securing nutritious and secure food supply is paramount. With the constant and aggravated depletion of our natural environments, such as water and soils supplies, the foundations of our food production are compromised. There is a demand for alternative food sources that also envision a sustainable way for the exploration of these foundations in which our food productions lie. In that scenario, there is a great interest in macroalgae biomass and resource exploration with a substantial body of evidence supporting macroalgae nutritious potential. Consumed for centuries in eastern countries, macroalgae consumption has gained interest in western societies due to their bioactive properties, awareness regarding nutritional food and overall sustainability in their production and consumption [1,2]. Macroalgae possess several advantages in comparison to land crops, with the most prevalent advantage being the independence of freshwater usage. However, such consumption requires further standardization and clearance. While land crops have been optimized for years and species/classes are legislated and safely consumed, consumption of macroalgae still faces legislative and food security barriers.

Additionally, it is still unclear which species should be nutritiously favored and methods for production are still far from optimized. Harvesting and consumption of wild macroalgae should be avoided, as these organisms have tremendous bioactive and intrinsic compound variability and are susceptible to environmental alterations and pollution.

This work focuses on the description and analysis of the bioactive and nutritional profiles of four macroalgae collected in the Western Portuguese coast (*Undaria pinnatifida*, *Osmundea pinnatifida*, *Fucus vesiculosus* and *Calliblepharis jubata*) regarding uronic and fatty acids, polysaccharides, pigments and phenolic content.

This can advance our understanding of nutritional composition of various macroalgae species, improving our efforts into production of species with the capability to offer nutritious alternatives.

Acknowledgments: The authors acknowledge the Centre for Functional Ecology—Science for People and the Planet (CFE; UIDB/04004/2020; <https://doi.org/10.54499/UIDB/04004/2020>), financed by FCT/ MCTES through national funds (PIDDAC) and the Associate Laboratory TERRA (LA/P/0092/2020; <https://doi.org/10.54499/LA/P/0092/2020>). This research was financed by the project MENU—Marine Macroalgae: Alternative recipes for a daily nutritional diet (FA_05_2017_011), funded by the Blue Fund under Public Notice No. 5-Blue Biotechnology and co-financed by the project AlgaMar4antivirus—Marine Macroalgae in the prevention and treatment of ANTiviral diseases (Coronavirus SARS-CoV-2) (FA_07_2018_009), funded by the Blue Fund under Public Notice No. 7/2018—Scientific Research and Marine Technology.

References:

- [1] Pereira, L., Cotas, J., Gonçalves, A.M., Seaweed Proteins: A Step towards Sustainability? *Nutrients*, 16 (2024) 1123.
- [2] Lomartire, S., Gonçalves, A.M.M., Algal Phycocolloids: Bioactivities and Pharmaceutical Applications. *Mar. Drugs*, 21 (2023) 384.

Design of innovative electrochemical genosensors for honey fraud and quality detection by botanical origin authentication

Stephanie L. Morais^{1,2}, Michelle Castanheira^{1,2}, Eduarda Pereira^{1,3}, Marlene Santos³, Valentina F. Domingues¹, Cristina Delerue-Matos¹, M. Fátima Barroso^{1,*}

¹ REQUIMTE|LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal

² Departamento de Química, Faculdade de Ciências, Universidade do Porto, Portugal

³ REQUIMTE|LAQV, Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal
* mfb@isep.ipp.pt

Ensuring food safety has become a concern for companies, consumers and the government alike, due to the increase of fraudulent and/or adulterated food products found in the global market [1,2]. Some common fraudulent strategies include mislabelling a product's geographical origin and blending it with lower-grade substances [3].

Among the frequently adulterated food products there is honey. Honey is a highly sought-after natural food with an elevated environmental, social and commercial value due to its rich nutritional profile and numerous health benefits. However, it is also vulnerable to adulteration [4]. Therefore, it is important to develop an analytical technique that can quickly, cheaply, and easily assess the quality and safety of honeys worldwide.

In this work, an electrochemical genosensor for the detection of two plant species: *Erica arborea* and *Castanea sativa* in real honey samples was designed and optimized. The first step was the construction of the genosensor. For this, DNA-target probes capable of unequivocally detecting *E. arborea* and *C. sativa* DNA were selected and designed. As a sandwich-format strategy was adopted, their complementary probes were then cut into two smaller sequences to which a thiol group (DNA-capture) and a fluorescein (DNA-signalling) were attached.

Using chronoamperometry, the enzymatic amplification of the electrochemical signal was achieved with a concentration range of 0.07 to 2.00 nM. These results were then compared to the DNA from certified *E. arborea* and *C. sativa* plant leaves, amplified by PCR, and 10 commercial honeys found in local Portuguese markets. The developed genosensor was successfully applied for the detection of the DNA from the extracted plant leaves and commercial honey samples. So, the developed genosensor is a promising cost-effective and innovative analytical method to detect the botanical origin of real honeys.

Acknowledgments: This work received financial support from national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through project MTS/SAS/0077/2020—"Honey+—New reasons to care honey from the Natural Park of Montesinho: A bioindicator of environmental quality & its therapeutic potential" and through the projects UIDB/50006/2020 and UIDP/50006/2020. MFB thanks Fundação para a Ciência e a Tecnologia (FCT) for the FCT Investigator (2020.03107.CEECIND). Stephanie Morais (2023.028929.BD), Eduarda Pereira (2024.01697.BDANA), Michelle Castanheira (2023.05159.BDANA) are grateful to FCT and the European Union (EU) for their grants financed by POPH-QREN-Tipologia 4.1-Formação Avançada, funded by Fundo Social Europeu (FSE) and Ministério da Ciência, Tecnologia e Ensino Superior (MCTES).

References:

- [1] Y.K. Anagaw, W. Ayenew, L.W. Limenh, D.T. Geremew, M.C. Worku, T.A. Tessema, W. Simegn, M.L. Mitku, *SAGE open medicine*, 12 (2024), 20503121241250184.
- [2] C. Brooks, L. Parr, J.M. Smith, D. Buchanan, D. Snoich, A. Hebishy, *Food Control*, 130 (2021) 108171.
- [3] L. Manning, J.M. Soon, *Journal of Food Science*, 81 (2016), 823-834.
- [4] S. Soares, F. Rodrigues, C. Delerue-Matos, *Molecules*, 28 (2023) 2106.

Assessing the nutritional potential of *Undaria pinnatifida* and *Himanthalia elongata* for human consumption

Aurora Silva^{1,2}, Cristina Soares^{1*}, Franklin Chamorro², Maria Carpena², Rafael Nogueira-Marques², M. Fátima Barroso¹, Miguel A. Prieto², Cristina Delerue-Matos^{1*}.

¹REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto.

² Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Instituto de Agroecoloxía e Alimentación (IAA) – CITE XVI, 36310 Vigo, Spain.

* cds@isep.ipp.pt

The world production of marine macroalgae, or seaweed, has more than tripled since the turn of the millennium, increasing from 10.6 million tonnes in 2000 to 32.4 million tonnes in 2018. Brown algae constitute a significant part of this production, with various species being cultivated and utilized across different countries [1]. For example, species such as *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina japonica*, *Undaria pinnatifida*, and *Himanthalia elongata* are widely cultivated and used for food, alginates, and other products. Some of these species are native to the Iberian Peninsula and can be found along the northern beaches of Portugal and Galicia [2]. The brown algae are also notorious for the bioactive secondary metabolites that are described as having antioxidant, antimicrobial and neuroprotective activities among others [3].

In this study, the species *Undaria pinnatifida* (UP) and *Himanthalia elongata* (HE) were analyzed to assess their nutritional profile, considering their lipid, amino acid, and mineral content. The proximate composition analysis revealed that both species contain approximately 20% mineral residue (ash). Their mineral profile is similar, with sodium levels around 35 g/kg of dried algae (dw) and potassium at approximately 80 g/kg dw. Calcium and iron were quantified at around 8 g/kg dw.

The protein content was determined to be 7% in HE and 10% in UP, while fiber content was approximately 30%. The total lipidic content was around 6% and the fatty acid profile revealed a predominance of palmitic acid and oleic acid.

Regarding amino acid composition, UP contains all essential amino acids except for phenylalanine, whereas HE lacks only valine. Both species exhibit significant amounts (ranging from 50 to 150 mg of amino acid per gram of protein) of aspartic acid, glutamic acid, and arginine. These results highlight the potential contribution of seaweeds to nutrient uptake in the context of a balanced diet.

Acknowledgments: This work received financial support from FCT/MCTES (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds and also to Ibero-American Program on Science and Technology (CYTED— GENOPSYSSEN, P222RT0117). Fatima Barroso (2020.03107.CEECIND) thank FCT for the FCT Investigator grant. Aurora Silva thanks EU-FORA Fellowship Program (EUBA-EFSA-2023-ENREL-01) for the grant and for the support of Frank Chamorro work.

References:

- [1] FAO; WHO Report of the Expert Meeting on Food Safety for Seaweed – Current Status and Future Perspectives; 2022; ISBN 9789251365908.
- [2] Pereira, L. Guia Ilustrado Das Macroalgas: Conhecer e Reconhecer Algumas Espécies Da Flora Portuguesa; Imprensa da Universidade de Coimbra, 2009; ISBN 9789892603971.
- [3] Silva, A.; Cassani, L.; Grosso, C.; Garcia-Oliveira, P.; Morais, S.L.; Echave, J.; Carpena, M.; Xiao, J.; Barroso, M.F.; Simal-Gandara, J.; et al. Recent Advances in Biological Properties of Brown Algae-Derived Compounds for Nutraceutical Applications. Crit. Rev. Food Sci. Nutr. 2024, 64, 1283–1311, doi:10.1080/10408398.2022.2115004.

Antioxidant potential of seaweed extracts in fish oil preservation

Francisca Santos¹, Cristina Soares^{1,*}, Valentina Domingues¹, Maria João Ramalhosa¹, Ana Cação², Helga Augusto², Cristina Delerue-Matos¹

¹REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal

²Cofisa, S.A, Figueira da Foz, Portugal

* cds@isep.ipp.pt

The deterioration of food quality and shelf life is a critical challenge in the food industry due to lipid peroxidation [1]. Lipid peroxidation is a chemical process that leads to the degradation of lipids, resulting in the formation of harmful compounds, undesirable flavors (rancidity), and decreased nutritional value [2,3]. Thus, this study evaluates the effectiveness of seaweed extracts as natural antioxidants in preventing lipid oxidation during the accelerated aging of oils typically used in canned fish.

Ten seaweeds were selected: four brown (*Fucus vesiculosus*, *Himanthalia elongata*, *Eisenia bicyclis*, and *Undaria pinnatifida*), two green (*Ulva lactuca* and *Codium tomentosum*), and four red (*Palmaria palmata*, *Gracilaria gracilis*, *Chondrus crispus*, and *Porphyra dioica*). Algal extracts obtained through subcritical water extraction (SWE) at 140°C [4] were evaluated for their antioxidant potential in sunflower oil. Accelerated aging tests were conducted at 50°C over 16 days, with aliquots collected every 4 days. Lipid oxidation was assessed by measuring the peroxide index (PI) as an indicator of primary oxidation products. The results were compared to a control sample (oil without antioxidants) and oil treated with butylated hydroxytoluene (BHT), providing an understanding of the effectiveness of the algal extracts in oxidative stability. *Palmaria palmata* is the best at preventing peroxide formation in this setup, with results even better than those obtained with BHT. *Gracilaria gracilis* showed the highest peroxide value, suggesting it is the least effective in preventing lipid peroxidation.

These findings indicate that seaweeds have the potential to serve as natural antioxidants for food applications and could potentially replace synthetic alternatives. Further research is necessary to optimize extract concentration, evaluate their nutritional impact, assess their effect on food quality, and investigate the long-term influence of seaweed extracts on the shelf life of canned fish stored in brine and oil.

Acknowledgments: This work has been developed within the scope of "BLUE BIOECONOMY INNOVATION PACT" (Project N°. C644915664-00000026) financed by NextGenerationEU, under the incentive line "Agendas for Business Innovation" of the Recovery and Resilience Plan (PRR). This work was supported by projects REQUIMTE/LAQV—UIDB/50006/2020, UIDP/50006/2020, and financed by FCT/Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through national funds.

References:

- [1] Othón-Díaz ED, Fimbres-García JO, Flores-Sauceda M, Silva-Espinoza BA, López-Martínez LX, Bernal-Mercado AT, et al. Antioxidants in Oak (*Quercus* sp.): Potential Application to Reduce Oxidative Rancidity in Foods. *Antioxidants* 2023;12. <https://doi.org/10.3390/antiox12040861>.
- [2] Domínguez R, Pateiro M, Gagaoua M, Barba FJ, Zhang W, Lorenzo JM. A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants* 2019;8. <https://doi.org/10.3390/antiox8100429>.
- [3] Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid Med Cell Longev* 2014;2014:1–31. <https://doi.org/10.1155/2014/360438>.
- [4] Santos F, Soares C, Morais SL, Neves C, Grosso C, Ramalhosa MJ, et al. Optimized Extraction Protocols for Bioactive Antioxidants from Commercial Seaweeds in Portugal: A Comparative Study of Techniques. *Foods* 2025;14:453. <https://doi.org/10.3390/foods14030453>.

Influence of drying method and packaging material on the shelf life of orange-fleshed sweet potato flour

Nelson Pereira^{1,2*}, Ana Cristina Ramos^{1,3}, Marco Alves⁴, Vítor D. Alves^{2,5}, Manuela Vida¹, Margarida Moldão^{2,5}, Marta Abreu^{1,2,5*}

¹ INIAV - Instituto Nacional de Investigação Agrária e Veterinária, Unidade de Tecnologia e Inovação, Oeiras, Portugal;

² LEAF - Linking Landscape, Environment, Agriculture and Food Research Center, Instituto Superior de Agronomia, ULisboa, Lisboa, Portugal;

³ GeoBioTec - Geobiociências, Geoengenharias e Geotecnologias, FCT-UNL, Caparica, Portugal;

⁴ INOV.LINEA/TAGUSVALLEY – Science and Technology Park, Abrantes, Portugal;

⁵ Associate Laboratory TERRA, Instituto Superior de Agronomia, ULisboa, Lisboa, Portugal.

* isa128286@isa.ulisboa.pt; marta.abreu@iniav.pt

Orange-fleshed sweet potato (OFSP) is renowned for its high nutrient and bioactive content, and its transformation into flour through drying is a key postharvest preservation strategy. However, the nutritional, bioactive, and functional quality of OFSP flour may deteriorate over time [1], prompting the development of sustainable packaging alternatives that balance preservation efficiency with environmental responsibility [2]. This study assessed the influence of packaging materials on the storage stability of hot-air-dried (75 °C/20h) and freeze-dried (-41 to 30°C/70h) OFSP flours. The flours were packaged in three heat-sealable materials (LDPE, PLA, and PET-Al) and stored at 25 ± 2°C for 6 months, with analysis conducted every 3 months for moisture content (MC), CIELab colour, total phenolic (TPC, Folin-Ciocalteu method), total carotenoid (TCC), and total anthocyanins (TAC) contents, antioxidant activity (AOx; DPPH and FRAP methods), water and oil absorption capacities (WAC and OAC), and microbiological counts. Packaging significantly influenced moisture uptake, with PET-Al maintaining MC below 7%, whereas PLA and LDPE allowed values above 12% and 8%, respectively. Hot-air-dried flours exhibited better colour preservation than freeze-dried counterparts, particularly in LDPE. Both drying methods showed a decline in TPC and FRAP over storage, regardless of packaging. TCC decreased in LDPE- and PLA-packaged hot-air-dried flours but remained stable in PET-Al-packaged flours. Conversely, freeze-dried flours exhibited a TCC increase in PET-Al, while LDPE- and PLA-packaged flours maintained the initial TCC. TAC and DPPH-DCM values remained unchanged across all packaging materials. Microbial counts decreased over time across all packaging materials and drying methods, remaining within tolerable safety limits [3]. Hot-air-dried flours exhibited significant increases in WAC and OAC, whereas freeze-dried flours maintained constant values, regardless of packaging. These findings indicated that freeze-drying combined with high-barrier PET-Al packaging effectively preserved OFSP flour quality for at least 6 months of storage, offering promising implications for reducing postharvest losses and promoting sustainable food systems.

Acknowledgements: The authors would like to thank NativaLand, Lda. (Portugal) for supplying the sweet potato and the technical team at the TagusValley pilot plant (INOV.LINEA) with valuable support in developing the drying experiments. National funds funded this work through FCT – Fundação para a Ciência e a Tecnologia, IP, under the projects UIDB/04129/2020 of LEAF – Linking Landscape, Environment, Agriculture and Food Research Center and UIDB/04035/2020 of GeoBioTec – Geobiociências, Geoengenharias e Geotecnologias and through doctoral grant 2023.00711.BDANA.

References:

- [1] D. Agarwal, L. Mui, E. Aldridge, J. McKinney, L. Hewson, and I. D. Fisk, "The progression of lipid oxidation, β -carotenes degradation and sensory perception of batch-fried sliced sweet potato crisps during storage," *Food Funct.*, vol. 12, no. 10, pp. 4535–4543, 2021,
- [2] F. Versino, F. Ortega, Y. Monroy, S. Rivero, O. V. López, and M. A. García, "Sustainable and Bio-Based Food Packaging: A Review on Past and Current Design Innovations," *Foods*, vol. 12, no. 1057, 2023,
- [3] M. Van Hal, "Quality of sweetpotato flour during processing and storage," *Food Rev. Int.*, vol. 16, no. 1, pp. 1–37, 2000,

Choux pastry - the role of extrusion for improved pre-gelatinised wheat flour

Luís Rocha^{1*}, Fátima Martins^{1,2}, Ana Paula Pereira^{1,2}, Isabel Reinas³, Filipa S. Reis², Cristina Caleja², Lillian Barros², Alexandre Gonçalves^{1,2}

¹MORE – Mountains of Research Collaborative Laboratory – Association, Bragança, Portugal

²CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal

³TECPAN - Tecnologia e Produtos para Pastelaria e Panificação Lda, Mirandela, Portugal

**lrocha@morecolab.pt*

Extrusion is a technological process that transforms and gives better specific characteristics, such as texture, flavour and appearance to food products. The raw materials most used in the extrusion process are starch and protein-based materials and mostly tend to be cereal flours. This process is important because, by altering the structure of the starch and proteins in the flour, improves food functional properties, guarantees increased digestibility, and increases food preservation and safety. In pastry, the use of wheat pre-gelatinized flour allows to eliminate the pre-cooking and cooling stage in choux preparations, facilitating the final product preparation with the consequent savings in costs and time.

This work, carried out in partnership with the company Tecpan, aimed to study different extrusion conditions to obtain a pre-gelatinized wheat flour with improved characteristics towards a commercial flour, used as reference. Extrusion was performed in a Process 11 twin-screw extruder (Thermo Scientific, Germany) equipped with a 2 mm die. Wheat flour was subjected to different extrusion conditions of barrel temperature (110-160 °C), speed (200-700 rpm), and water content (12-33 %). The different extruded samples obtained were ground and physicochemical characterized according to water binding capacity, water solubility index, percentage of organic matter and ash, as well as granulometry and viscosity. The pre-gelatinized wheat flours' performance was validated on a choux pastry preparation, known as profiteroles. The profiteroles developed were characterized in terms of its inner expansion and volume. The results indicated that the process of extrusion increased water absorption, water solubility, and viscosity of wheat flour. Additionally, different extrusion conditions promote the production of pre-gelatinized flours with distinct physic-chemical properties, impacting the profiteroles manufacturing process and quality characteristics. In sum, the conditions applied in the extrusion process must be carefully studied to obtain improved pre-gelatinized wheat flour for pastry applications.

Acknowledgments: This work and Cristina Caleja, L. Rocha and F. Martins contracts were supported by national funds through Agenda VIIFOOD – Plataforma de Valorização, Industrialização e Inovação Comercial para o AgroAlimentar (no. C644929456-00000040), supported under the PRR (www.recuperarportugal.gov.pt) and financed by the European Union/Next Generation EU. This work was also financially supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020). The authors also thank the National funding by FCT, through the institutional scientific employment program contract with L. Barros (DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002), and the individual scientific employment program contract with F.S. Reis (2021.03728.CEECIND). A.P. Pereira and A. Gonçalves contracts were supported by PRR - Investment RE-C05-i02: Interface Mission - CoLAB- 01/C05-i02/2022.

Conception, development and construction of an Industrial-scale pilot-plant for desinfestation of chestnuts

**Natércia Fernandes^{1,2,*}, Elisabete Alexandre³, Carlos Freire³, Ângela Fernandes²,
Luís Rocha¹, Lillian Barros², Alexandre Gonçalves^{1,2}**

¹MORE – Laboratório Colaborativo Montanhas de Investigação – Associação, Bragança, Portugal

²CIMO, LA SusTEC, Instituto Politécnico de Bragança, Bragança, Portugal

³PERFINOX – Indústrias Metalúrgicas SA, Mansores, Portugal

*nfernandes@morecolab.pt

Portugal plays a worldwide significant role in the sector of chestnuts (*Castanea sativa* Mill). In spite of its modest geographical area, Portugal occupies the second position among European countries as producer and also as exporter of chestnuts. However, considerable economical losses still occur due to the common infestation of chestnuts by insects (weevil, *Curculio elephas*, and moths, mostly *Cydia splendana*). Their larvae gradually excavate galleries in the ripe fruit matrix, leading to its destruction. Moreover, chestnuts infested by insects are also more prone to any subsequent attack of fungi. Some chemical solutions have been used in the past to cope with the problem, but more recently they have been forbidden by stricter European legislation based on health, ecological, and safety reasons.

In this context, and in the scope of A2.4 – *New Post-harvest technologies for chestnuts* which belongs to the VIIAFOOD Agenda, the disinfestation problem is under study in order to evaluate if the radio-frequency technology can be used to disinfest chestnuts in a post-harvest scenario, that is, at the processing plant site. With that purpose and so far, an industrial-scale pilot-plant for applying electromagnetic waves within the radio-frequency range has been conceived, designed and built. The machine is about 6m long and 1m wide. It is equipped with 4 electromagnetic applicators, each of them connected to an independent radio wave generator and matching box. A conveyor moves the chestnuts into the applicators, where they are exposed to the electromagnetic fields. The machine has a continuous mode of operation and is equipped with a graphical user interface for human-machine interaction (HMI). It also has a simple refrigerating system to keep the temperature of the main electric components at safe levels.

This poster encompasses the building of such machine and presents its main characteristics and features.

Acknowledgments: This work was carried out under Agenda VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação Comercial para o AgroAlimentar (reference C644929456-00000040), which has the financial support of the European Union/Next Generation EU via the national Programa de Recuperação e Resiliência (PRR) (www.recuperarportugal.gov.pt).

This work was also supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020). The authors also thank the national funding by FCT, through the institutional and individual scientific employment program contract with L. Barros (DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002) and A. Fernandes (2023.08113.CEECIND) contracts, respectively.

Genopheno4trait project - genomic and phenotypic traits contributing to persistence of *listeria monocytogenes* in food processing environment

Lúcia Noronha¹, Ana Sousa¹, Vânia Borges², Rui Magalhães², Paula Teixeira²

¹Colab4Food - Collaborative Laboratory for Innovation in the Agri-Food sector, Rua dos Lagidos, 4485-655 Vairão

²Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto

Listeria monocytogenes is a foodborne pathogen responsible for a deadly disease - listeriosis- and major food recalls. Listeriosis represents the most severe human infectious disease in terms of hospitalization, with the highest case fatality rate of zoonotic diseases in the EU. In addition to suffering, functional disability and death, *L. monocytogenes* have a huge economic impact in food industry. Some strains of this foodborne pathogen can persist for months to years in Food Processing Environment (FPE), affecting all the food chain. These strains have a major role in contamination events, and consequently major impact in food industry.

The overall objective of the GenoPheno4Trait project is to improve food safety and decrease food waste reducing food contamination with *L. monocytogenes*. This project addresses the control of *L. monocytogenes* along the food chain contributing to support human health through reducing listeriosis. The proposed holistic approach will augment knowledge and define control strategies regarding persistence of *L. monocytogenes* in the FPE.

Keywords: *Listeria monocytogenes*; Food Processing Environment; Food Safety; Biocontrol strategies





Nutrition, Health & Wellbeing

Fortification of White Wine Vinegar with Anthocyanin-Rich Edible Flowers: Effects on Polyphenol Content, Aromatic Profile, and Sensorial Properties

**Margarida Teixeira^{1,*}, Alessandra Rinaldi², Nuno Mateus¹, Victor de Freitas¹,
Hélder Oliveira¹**

¹ REQUIMTE/LAQV, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Porto, Portugal

² Better Sensing, Terinov - Parque de Ciência e Tecnologia da Ilha Terceira, Terra Chã, Angra do Heroísmo 9700-702 Terra Chã

* up202103465@edu.fc.up.pt

Edible flowers (EFs) have been part of the human diet for centuries. With globalization and growing consumer awareness, the promotion of healthy diets has become a central theme in recent years, blending traditional lifestyles with emerging trends [1]. Anthocyanin-rich EFs not only provide vibrant colors but are also associated with health benefits [2, 3]. Their incorporation into food matrices, holds great potential to enhance their phytochemical composition, creating functional and innovative products aligned with consumer demand for healthier, sustainable options [4].

This work explored the incorporation of EFs into white vinegar on phytochemical content, aromatic profile, physicochemical and sensorial properties. The anthocyanin and polyphenol content of wild pansy, cosmos, and cornflower EFs, along with their fortified vinegars, were determined. Violanthin, apigenin-7-O-glucuronide, apigenin-7-(feruloyl)-glucoside and phenylacetic acid were the main polyphenols identified on wild pansy, cosmos, cornflower and control vinegars, respectively. Total anthocyanin and polyphenol content (TAC and TPC), antioxidant activity, pH, and color evolution were evaluated over a month. Cosmos vinegar showed the greatest color variation, and TPC increased in all fortified vinegars, with significant differences for wild pansy and cosmos. Wild pansy vinegar exhibited the highest antioxidant activity in DPPH assay. The aromatic profile of EFs and fortified vinegars, determined by GC-MS, identified 3-methylbutanoic acid, phenylethyl alcohol and nonanoic acid as the most abundant volatile compounds. Lastly, a sensorial analysis with a questionnaire was performed for both EFs and the vinegars and revealed cosmos vinegar as the most appreciated overall, followed by wild pansy and cornflower vinegar, with a similar trend observed for the EFs alone.

These findings demonstrate that incorporating EFs into vinegars enhances their bioactive, aromatic and sensory attributes, highlighting their potential for fortifying other food matrices and creating diverse, phytochemically rich, and bioactive products with potential health benefits.

Acknowledgments: The authors acknowledge the national funds provided to the development of this work, by Fundação para a Ciência e Tecnologia (FCT) under the scope of the project “AnthoE.Flos” (2022.01014.PTDC). MT also acknowledges her FCT PhD grant (2024.01090.BD).

References:

- [1] Teixeira, M., et al., Trends in Food Science & Technology, 2023. **138**: p. 708-725.
- [2] Cosme, P., et al., Antioxidants, 2020. **9**(12): p. 1263.
- [3] He, J., et al., Comprehensive Reviews in Food Science and Food Safety, 2022. **21**(4): p. 3096-3128. [4] Sik, B., et al., Food Science & Nutrition, 2024. **12**(5): p. 3265-3272.

Nutritional and Contaminant Analysis of Foods Ingested by Vegetarian Consumers: Total Diet Study Approach

Marta Ventura^{1, 2*}, Sandra Gueifão¹, Andreia Rego¹, Inês Delgado¹, Inês Coelho¹

¹ Department of Food and Nutrition, National Institute of Health Doutor Ricardo Jorge (INSA IP), 1649-016 Lisboa, Portugal

² MARE - Marine and Environmental Sciences Centre, Dep. de Ciências e Engenharia do Ambiente, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Costa de Caparica, Portugal

* *marta.ventura@insa.min-saude.pt*

Dietary habits have changed over the years, with the popularity of vegetarian and vegan diets increasing [1]. Total Diet Studies (TDS) are a public health tool for assessing dietary exposure to contaminants and beneficial substances in foods [2]. This study aimed to determine the profile of micronutrients and inorganic contaminants in the Portuguese population's most commonly consumed plant-based foods. The sampling plan followed the TDS methodology, and a total of five main food groups were analysed: grains and grain-based products (n=48); milk and dairy products (n=60); products for non-standard diets, food imitates and food supplements (n=72); pulses, dried fruits, oilseeds and spices (n=132); and vegetables and vegetable products (n=12). The 324 individual samples collected were analysed as 27 pooled samples. Each sample was analysed by Inductively Coupled Plasma Mass Spectrometry for thirteen elements: arsenic, cadmium, chromium, cobalt, copper, iodine, lead, lithium, manganese, molybdenum, selenium, strontium, and zinc.

The analytical results were obtained under quality assurance conditions supported by the requirements described in NP ISO/IEC 17025:2017.

The results of this study indicated that some food samples from the group of pulses, dried fruits, oilseeds and spices had the highest content of manganese, zinc, molybdenum and selenium. On the other hand, products for non-standard diets and dairy products had the highest content of copper and iodine, respectively. The arsenic, cadmium, and lead levels were below the limit of quantification for the majority of samples analysed.

The results demonstrated that while the consumption of 100 g of foods, like chia seeds, textured soy protein, and Brazil nuts, significantly exceeds the Dietary Reference Value for essential elements like copper, manganese, molybdenum, and selenium, others, particularly within dairy products, fall short of these nutritional benchmarks. This study revealed that the levels of inorganic contaminants (arsenic, cadmium and lead) are safe for the Portuguese population in the analysed samples.

References:

- [1] Melina, V., Craig, W., & Levin, S. (2016). Position of the Academy of Nutrition and Dietetics: Vegetarian Diets. *Journal of the Academy of Nutrition and Dietetics*, 116(12), 1970–1980. <https://doi.org/10.1016/j.jand.2016.09.025> X. Surname, Y. Surname, Z. Surname, *Journal name*, 12 (2019) 123.
- [2] Moy, G. G. (2013). *Total Diet Studies*. Springer New York Heidelberg Dordrecht London.

Fostering Smart Nutrition: mitigating the effects of child food insecurity

Ana Costa e Silva¹; Inês Baía Dias¹; Mariana Amorim²; Sílvia Fraga²; Elisabete Ramos², Marlene Sousa¹

¹ ProChild CoLAB Against Child Poverty and Social Exclusion, R. de Vila Flor 166, 4810-225 Guimarães, Portugal.

² EPIUnit ITR, Instituto de Saúde Pública da Universidade do Porto, Universidade do Porto, Rua das Taipas, n° 135, 4050-600 Porto, Portugal
ana.silva@prochildcolab.pt

Background: Food insecurity (FI) represents a significant public health challenge with adverse effects on the health and development of children and families. The Fostering Smart Nutrition (FSN) program aimed to mitigate these effects through a community-based participatory approach, focusing on two core interventions: food literacy workshops and the "Super Nutri" mobile application.

Methods: The food literacy workshops were targeted at children and caregivers to improve their knowledge and promote skills on healthy and sustainable food selection, preparation, and consumption. The six workshops were co-developed using insights from focus groups with caregivers, children, and educators and included interactive teaching methods during one year. The topics covered included nutrition, food budgeting, and reducing food waste, aiming to foster informed and healthier dietary choices and sustainable food practices. Complementing the workshops, the "Super Nutri" mobile application was developed to provide personalized, budget-friendly meal suggestions based on available ingredients. The app works offline and integrates educational content from the workshops and practical guidance on meal preparation and reduction of food waste.

Results: Initial data from the FSN program, involving 50 children aged 6-12 years from two socially vulnerable neighborhoods in northern Portugal, show that 48% of the households experience FI. Additionally, a significant proportion of children display poor dietary behaviors, including high sugar consumption (46%) and low intake of fruits and vegetables (56%). Preliminary data from the postintervention evaluation indicate improvements in food literacy and positive feedback from participants, suggesting increased awareness and adoption of healthier food practices.

Conclusions: These preliminary findings suggest that community-based food literacy interventions, supported by digital tools, may mitigate the negative consequences of food insecurity and improve dietary habits in vulnerable populations. Further research should evaluate long-term effects of these interventions on food security and health outcomes.

Acknowledgments: The authors would like to express their gratitude to the to the Laboratório da Paisagem team (Carlos Ribeiro, Susana Falcão, Daniel Ferreira) and to the Porta 7 team (Rita Magalhães, Mariana Silva, Mariana Costa, Sofia Machado) for their support and dedication to the field work.

References:

- [1] FAO, IFAD, UNICEF, WFP and WHO. The State of Food Security and Nutrition in the World 2023. Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum. Rome, FAO, 2023. <https://doi.org/10.4060/cc3017en>
- [2] C. M. Pollard, & S. Booth. Food Insecurity and Hunger in Rich Countries—It Is Time for Action against Inequality. International Journal of Environmental Research and Public Health, 2019.
- [3] <https://doi.org/10.3390/IJERPH16101804>

From carob pulp to cereal bowls: A functional flour for wellness and sustainability

Sofia M. Amoedo¹, Daniela A. Gonçalves¹, José A. Teixeira^{1,2}, Clarisse Nobre^{1,2,*}

¹ Centre of Biological Engineering (CEB), University of Minho, Braga, Portugal

² Associate Laboratory (LABBELS), Braga, Guimarães, Portugal

* clarissenobre@ceb.uminho.pt

Background: The growing demand for low-sugar functional foods reflects a shift toward health-conscious lifestyles. Prebiotics like fructo-oligosaccharides (FOS) offer a promising approach to modulating microbiota for disease prevention [1]. Carob pulp, with sucrose and polyphenol richness, serves as a valuable substrate to produce FOS, with intrinsic health benefits.

Aim: This study aimed to create a sustainable functional food product from carob pulp by converting its intrinsic sucrose content into FOS and enhancing its bioactive properties [2].

Methods: Two approaches were utilized to synthesize FOS from carob pulp extracts: (1) enzymatic hydrolysis using Viscozyme® L, Pectinex® Ultra SP-L, Catazyme® 25 L, and Novozym® 960; and (2) fungal fermentation with *Aureobasidium pullulans* CCY 27.1.94 and *Aspergillus ibericus* MUM 03.49.

Results: Optimal sucrose extraction was achieved using a pulp-to-water ratio of 1:3 (w/v) at 46.1 °C for 132 minutes, yielding 86 ± 1 g/L of sucrose. Novozym® 960 generated the highest FOS yield (0.423 ± 0.013 g_{FOS}/g_{initial sucrose}) with a purity of $29.6 \pm 0.3\%$, while *A. ibericus* achieved a yield of 0.347 ± 0.003 g_{FOS}/g_{initial sucrose}. The carob pulp extract demonstrated significant antioxidant activity (72.1 ± 0.2 μmol TEq/g DW via FRAP and 5.35 ± 0.05 μg/mL via ABTS), along with high phenolic (8.57 ± 0.04 mg GAEq/g DW) and flavonoid (2.74 ± 0.02 mg CEq/g DW) content. Treatment with Novozym® 960 enhanced the release of bioactive compounds, further increasing antioxidant activity, whereas fungal fermentation modified or reduced these phytochemicals. The FOS produced exhibited strong resistance to gastrointestinal digestion, with hydrolysis rates remaining below 10%.

Conclusions: Carob pulp presents significant potential as a sustainable and clean-label alternative for producing functional foods enriched with prebiotic FOS, phenolic acids, and flavonoids, which have been shown to provide antioxidant, anti-inflammatory, and anti-carcinogenic benefits [3]. A promising application involves incorporating FOS-enriched carob pulp flour into breakfast cereals, an approach that is currently being optimized.

Acknowledgments: This work was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020, and by Agenda VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação comercial para o Agroalimentar (n.º C644929456-00000040) with the project A 1.9 “Development of healthier and more sustainable food products under the scope of Programa de Recuperação e Resiliência (PRR) and NextGeneration EU European Fund. Daniela A. Gonçalves and Clarisse Nobre acknowledge the FCT for the PhD Grant (2022.11590.BD) and for the Assistant Research contract (DOI 10.54499/2021.01234.CEECIND/CP1664/CT0019), respectively.

References:

- [1] D. Gonçalves, V. Alves, J. Teixeira, C. Nobre, Food Research International, 168 (2023) 112671.
- [2] D. Gonçalves, J. Teixeira, C. Nobre, Future Foods, 9 (2024) 100387.
- [3] M. Rodríguez-Daza, E. Pulido-Mateos, J. Lupien-Meilleur, D. Guyonnet, Y. Desjardins, D. Roy, Frontiers in Nutrition, 8 (2021).

Promising Buccal Films Containing *Actinidia Arguta* Fruit Extract for Oral Mucositis Treatment: Characterization and *In Vivo* and *Ex Vivo* Permeation Profile

Filipa Teixeira¹, Ana Margarida Silva¹, Catarina Macedo¹, Berta Estevinho^{2,3}, Stefania Sut⁴, Stefano Dall'Acqua⁴, Cristina Delerue-Matos¹, Paulo C. Costa^{5,6}, Francisca Rodrigues^{1*}

¹REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Porto, Portugal

²LEPABE, Faculty of Engineering, University of Porto, Porto, Portugal

³ALICE, Faculty of Engineering, University of Porto, Porto, Portugal

⁴Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

⁵REQUIMTE/UCIBIO, MedTech-Laboratory of Pharmaceutical Technology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

⁶Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, Porto, Portugal

*francisca.rodrigues@graq.isep.ipp

Oral mucositis (OM) is a frequent adverse effect of cancer treatments such as chemotherapy, marked by the breakdown of oral mucosa integrity, inflammation, and pain [1,2]. Current methods to prevent and treat OM are inadequate, prompting the search for new active compounds, especially from natural sources like *Actinidia arguta* fruits, commonly designed as kiwiberry [1]. This fruit is well known for its therapeutic properties and health benefits, particularly the antioxidant, anti-inflammatory, and anticancer effects [3] attributed to the rich content in phenolic compounds, vitamins, and organic acids [3,4].

This study aimed to create buccal films containing *A. arguta* fruit extract as active ingredient to alleviate the OM symptoms. The buccal films were produced by solvent casting, using 1% HPMC K100 LV EP, 2.5% glycerin, and *A. arguta* extract, which was prepared via Ultrasound-Assisted Extraction [3,4]. The films were evaluated for several parameters including physical characteristics (weight: 194.8 mg; thickness: 0.37 mm; disintegration time: 15.05 min; moisture content: 10.53%; swelling capacity: 55.95%), mechanical properties (resistance to extension: 10.11 N; elongation percentage: 36.10%;

Young's modulus: 3440 MPa), and antioxidant activities (TPC: 6.46 mg GAE/g film; FRAP: 49.45 µmol FSE/g film; ABTS: 3.74 mg AAE/g film; DPPH: 4.90 mg TE/g film). *In vitro* 2D cell assays showed no adverse effects on HSC-3 and TR146 oral cell lines. Crucially, the compounds release profile was examined using *in vitro* 3D cell models (TR146) and *ex vivo* models with porcine mucosa (Franz diffusion cells) combined with LC/DAD-ESI-MS quantification. The findings indicated significant permeation of rutin (88.32%), quercetin-3-O-glucoside (84.95%), and catechin (79.74%). These results underscore the substantial potential and safety of buccal films with *A. arguta* fruit extract for the prevention of OM.

Acknowledgments: This work received financial support from project EXPL/BAA-GR/0663/2021 – Kiwi4Health: Exploring the Eco-Innovative Re-Use of Kiwiberry and the projects REQUIMTE/LAQV - UIDB/50006/2020, UIDP/50006/2020, and LA/P/0008/2020. Filipa Teixeira is thankful for the scholarship from project EXPL/BAAGR/0663/2021. Ana Margarida Silva (SFRH/BD/144994/2019) and Catarina Macedo (2022.12642.BD) are thankful for their Ph.D. grants financed by POPH-QREN and subsidized by the European Science Foundation and Ministério da Ciência, Tecnologia e Ensino Superior. Francisca Rodrigues (2023.06819.CEECIND) is thankful for her contract financed by FCT/MCTES—CEEC Individual Program Contract.

References:

- [1] A.S. Ferreira, C. Macedo, A.M. Silva, C. Delerue-Matos, P. Costa, F. Rodrigues, International Journal of Molecular Sciences, 23 (2022) 4385.
- [2] C. Pulito, A. Cristaudo, C.L. Porta, S. Zapperi, G. Blandino, A. Morrone, S. Strano, Journal of Experimental & Clinical Cancer Research, 39 (2020) 210.
- [3] C. Macedo, A.M. Silva, A.S. Ferreira, M.d.I.L. Cádiz-Gurrea, Á. Fernández-Ochoa, A. Segura-Carretero, C. Delerue-Matos, P. Costa, F. Rodrigues, Scientia Horticulturae, 313 (2023) 111910.
- [4] F. Teixeira, A.M. Silva, C. Macedo, B. Estevinho, S. Sut, S. Dall'Acqua, C. Delerue-Matos, P. Costa, F. Rodrigues, Journal of Drug Delivery Science and Technology, 96 (2024) 105725.

In vitro* evaluation of the antibacterial activity of putative probiotic strains against enterotoxigenic *Escherichia coli

Ana Rita Silva^{1*}, Piet Cools², Aliona Rosca², Nuno P. Mira^{3,4}, Carina Almeida^{1,5,6,7}, Joana Castro^{1,7}

¹Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV, I.P), 4485-655 Vila do Conde, Portugal

²Department of Diagnostic Science, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

³iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, 1049-001 Lisboa, Portugal

⁴Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

⁵LEPABE— Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁶ALiCE— Laboratório Associado para a Inovação em Engenharia Química, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁷Centro de Engenharia Biológica, Universidade do Minho 4710-057 Braga, Portugal

* joana.castro@iniav.pt

Swine enteric colibacillosis is caused by enterotoxigenic *Escherichia coli* (ETEC). The disease affects piglets during the neonatal period or post-weaning stage, resulting in dehydration, diarrhoea and, in severe cases, mortality. Previous treatments included antibiotics and zinc oxide, which was banned for use in 2022. The increasing resistance of bacteria to antibiotics and heavy metals has prompted researchers to investigate alternative therapies for the prevention and treatment of enteric colibacillosis, including probiotics. In this study, we aimed to determine the antimicrobial activity of probiotics and their cell-free supernatants (CFS) against ETEC infection. Thus, the anti-ETEC potential of probiotics candidates (n=31) was evaluated using the spot test agar. For the evaluation of the antimicrobial activity of CFS against ETEC, it was used a well diffusion assay. The hemolytic activity and auto-aggregation capacity of probiotic strains were also evaluated. Our findings demonstrated that all the putative probiotics examined here showed somewhat effect in inhibiting the growth of ETEC, through both contact-dependent (live cells) and contact-independent (CFS) interactions. However, the efficacy of different probiotics was found to vary depending on the ETEC strains (SP11 and SP31). Through the contact-dependent interactions, the greatest zone of inhibition (ZOI) against ETEC strains SP11 and SP31 was exhibited by *L. acidophilus* ATCC 4356 and *P. acidilactici* CNCTC 6986, respectively. When testing the CFS derived from putative probiotics, we observed that the most promising value of ZOI was obtained by *L. plantarum* FW0BV0887 against both ETEC SP11 and ETEC SP31. The majority of the tested probiotic candidates demonstrated γ -hemolysis or no hemolytic activity. Some strains exhibited high auto-aggregation, with the highest reaching approximately 46%, while others showed moderate to low values. In conclusion, the study underscores the potential of probiotics as effective alternatives to traditional treatments for mitigating ETEC infection in swine.

Acknowledgments: This work was financially supported by: APTAcoli (PTDC/CVT-CVT/4620/2021), funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI); national funds through FCT/MCTES (PIDDAC): LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020), project UIDB/04565/2020 of the Research Unit Institute for Bioengineering and Biosciences—iBB; project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB, and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020), and by FCT under the scope of the strategic funding of UIDB/04469/2020 unit (CEB). J.C. thanks FCT for the CEEC Individual (<https://doi.org/10.54499/2022.06886.CEECIND/CP1737/CT0001>).

***In vitro* digestion and bioaccessibility studies of Vitamin D3 in Chitosan/Gum Arabic particles' stabilized 3D printable Pickering Emulsions**

Lucas Shinti Iwamura¹, Liandra Gracher Teixeira¹, Maria Filomena Barreiro¹, Arantzazu Santamaria-Echart^{1*}

¹ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300- 253 Bragança, Portugal.

*asantamaria@ipb.pt

Vitamin D3 (cholecalciferol) is a bioactive compound essential for calcium absorption and bone health whose delivery in aqueous food systems is limited due to its hydrophobicity, together with other constraints, including light and temperature sensitivity [1]. Pickering emulsions (PEs), stabilized by solid particles, offer a surfactant-free, and eco-friendly, stable encapsulation alternative to address this challenge [2]. Also, Pickering emulsions printability enable the development of 3D printed products, offering innovative solutions in personalized foods, such as patients with dysphagia [3].

This study developed the encapsulation of vitamin D3 (1% g/L) in PEs stabilized by chitosan/gum Arabic nanoparticles (1:1, 1.5% w/v) and incorporated into foods structured through ionotropic gelation with calcium alginate (calcium source). The PEs were prepared with Miglyol 812N oil and homogenized in an Ultraturrax DI 25. The structured food was tested for its 3D printing capability using an extrusionbased bioprinter, aiming at applications in personalized nutrition. The encapsulation efficiency (EE) and vitamin D3 bioaccessibility were evaluated using an *in vitro* digestion method, simulating the oral, gastric, and intestinal phases.

The EE reached 89.7%, demonstrating the protective effect of the vitamin in the Pickering emulsions. The bioaccessibility decreased throughout the digestion phases: 71.57% in the oral phase, 62.6% in the gastric phase, and 19.62% in the intestinal phase, likely due to micelle aggregation limiting vitamin D3 absorption. Nevertheless, the Pickering emulsion maintained its stability under gastric conditions, protecting the vitamin D3 and preventing its premature degradation.

This study highlights the potential of chitosan/gum Arabic-stabilized Pickering emulsions for vitamin D3 encapsulation in functional foods. These emulsions offer a promising strategy for improving the delivery of lipophilic nutrients. Optimizing the formulation and the 3D-printed structure could enhance the intestinal absorption capacity, making this approach viable for functional food applications.

Acknowledgments: This work was financed by the Portuguese Foundation for Science and Technology (FCT), by the national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., in the framework of the project 2022.01308.PTDC (NutriPick3D) (DOI: 10.54499/2022.01308.PTDC); This work was supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI:10.54499/LA/P/0007/2020). FCT the scientific employment contract (A. Santamaria-Echart).

References:

- [1] M. Guttoff, A. H. Saberi, & D. J. McClements, Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry*, 171, 117–122 (2015). <https://doi.org/10.1016/j.foodchem.2014.08.087>
- [2] X. Yin, J. Lu, W. Du, Q. Wu, L. Han, S. Su, Encapsulation of β -carotene in Pickering emulsions stabilized by self-aggregated chitosan nanoparticles: Factors affecting β -carotene stability, *Int J Biol Macromol* 277 (2024) 133696. <https://doi.org/10.1016/j.ijbiomac.2024.133696>.
- [3] J. Hou, M. Liu, Y. Liu, R. Chuang, H. Zhang, L. Zheng, H. Li, N. Xia, Y. Ma, A.M. Rayan, M. Ghamry, Strategy to kill two birds with one stone: High internal phase Pickering emulsions to modulate 3D printed pork texture as a dysphagia diet, *Food Chem* 463 (2025) 141319. <https://doi.org/10.1016/j.foodchem.2024.141319>.

Polyphenols as enhancers of emulsifying and antioxidant properties of egg and yeast protein emulsions: understanding the molecular interactions behind their technological and functional potential

Ana Catarina Ribeiro¹, Carlo Bravo^{1,*}, Rui Miguel Ramos¹, Ricardo Dias¹, Nuno Mateus¹, Víctor de Freitas¹, Rosa Pérez-Gregorio^{1,2,3}, Susana Soares^{1,*}

¹REQUIMTE, LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal

² Food and Health Omics Group, Food and Agroecology Institute, University of Vigo, Campus As Lagoas, s/n, 32004 Ourense, Spain

³ Food and Health Omics Group, Department of Chemistry and Biochemistry, Galicia Sur Health Research Institute (IISGS), SERGAS-UVIGO, 32002 Ourense, Spain

* carlo.bravo@fc.up.pt; susana.soares@fc.up.pt

Alternative protein sources, such as yeast protein extracts (YPE), are gaining popularity due to their sustainability, affordability, and health benefits compared to animal-based proteins¹. However, their limited emulsifying capacity and off-flavors have hindered widespread adoption. In this context, polyphenols have emerged as a promising strategy to enhance the techno-functional properties of alternative proteins due to their natural ability to bind proteins².

This study investigates the molecular interactions between polyphenols and two dietary proteins, exploring how these interactions can be optimized through technological processes to improve the functional properties of polyphenol-protein complexes in mayonnaise-based emulsions³. Specifically, we examined the interactions between two polyphenol-rich extracts (PEs)—green tea extract (GTE), rich in flavanols, and blueberry extract (BE), rich in anthocyanins—and two model proteins: enolase (ENO), representing YPE, and ovalbumin (OVA), from egg white.

Spectroscopic analyses (UV-Vis, FTIR, and fluorescence) revealed significant alterations in the secondary and tertiary structures of the proteins, particularly under acidic conditions (pH 3.5). Additionally, these polyphenol-protein interactions enhanced the emulsifying properties of both ENO and OVA while conferring antioxidant activity, as demonstrated by square-wave voltammetry.

Acknowledgments: This work received financial support from FCT/MCTES (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds. The authors thank Cláudia Alves (FCUP| DQB-Lab&Services) for the technical assistance with FTIR experiments and the Science and Technology Foundation (FCT) for financial support of the research contract of Ana Catarina Ribeiro PhD fellowship (UI/BD/153612/2022). Rosa PérezGregorio thanks the National Agency for Research (AEI) from the Ministry of Science and Innovation for the “Ramón y Cajal” contract reference RYC2021-033224-I, which was funded by EU funds through the National Plan for Scientific Research and Innovation and the Program to Attract and Retain Talent.

References:

- [1] Ma, J. *et al.* Yeast proteins: The novel and sustainable alternative protein in food applications. *Trends in Food Science & Technology* **135**, 190-201 (2023).
- [2] Ribeiro, C. *et al.* in *Extraction, Characterization, and Functional Assessment of Bioactive Compounds* 65-75 (Springer, 2024).
- [3] Ribeiro, A. C. *et al.* Unravelling the effect of phenolic compounds in the design of a yeast protein-based emulsions. *International Journal of Food Science & Technology* **58**, 3337-3348 (2023).

Insects as a novel and functional food source to prevent hypertension: the case of house cricket

Carla S.S. Teixeira^{1,*}, Bruno Carriço-Sá¹, Rita Biltres¹, Caterina Villa¹, Joana Costa¹, Sérgio F. Sousa², Tânia G. Tavares^{3,4}, Miguel A. Faria¹, Isabel M.P.L.V.O. Ferreira¹, Isabel Mafra¹

¹ REQUIMTE-LAQV, Faculty of Pharmacy, University of Porto, Porto, Portugal

² REQUIMTE-LAQV, BioSIM, Faculty of Medicine, University of Porto

³ LEPABE, Faculty of Engineering, University of Porto, Porto, Portugal

⁴ ALiCE, Faculty of Engineering, University of Porto, Porto, Portugal

* cteixeira@ff.up.pt

Entomophagy, the practice of eating insects, has gained prominence in recent years as a nutritious, environmentally and economically beneficial alternative to meat protein [1]. However, the introduction of insects for human consumption in Western countries is facing some resistance mainly due to cultural issues. Insects are a good source of nutrients, with a high percentage of high-quality proteins that, when digested, can generate bioactive peptides with beneficial physiological effects [2]. Some peptides mediate their bioactivity by inhibiting target enzymes, such as dipeptidyl peptidase IV (DPP-IV) and somatic angiotensin-converting enzyme (sACE), which are used as modern therapeutic approaches for diabetes and hypertension prevention, respectively. According to the WHO, >1.13 billion adults have hypertension and >422 million people live with diabetes (95% with type 2). The purpose of this study is to present scientific evidence to support the aforementioned benefits and to disseminate this information to potential stakeholders and general public.

Firstly, an *in silico* approach was employed to identify bioactive peptides resulting from the simulated gastrointestinal digestion of proteins from *Acheta domesticus* (house cricket) [3], one of the four species regulated by the European Union as novel foods (Regulation (EU) 2022/188; Regulation (EU) 2023/5). This approach led to the identification of a group of 3 peptides, which were subsequently identified by LC-MS/MS in the *in vitro* digesta of *A. domesticus* and their sACE inhibitory capability demonstrated *in vitro* [4]. While further validation *in vivo* is still required to substantiate the antihypertensive capabilities of the peptides, the present findings indicate the potential health benefits of entomophagy and underscore the efficacy of *in silico* methodologies to predict bioactive peptides derived from food sources.

These findings may contribute to a paradigm shift in consumer perception regarding insect consumption. This is particularly relevant in the current era where information is readily available, and marketing has a significant influence on consumer behaviour.

Acknowledgments: This work received financial support from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020); project PTDC2022.05853 – SeaProPep, with DOI 10.54499/2022.05853.PTDC (<https://doi.org/10.54499/2022.05853.PTDC>), funded by national funds through FCT/MCTES (PIDDAC); LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020), and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020) funded by national funds through FCT/MCTES (PIDDAC). C. Villa, I. Mafra, and J. Costa thank FCT for funding through the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03670.CEECIND/CP1662/CT0011 and 2021.03583.CEECIND/CP1662/CT0012, respectively). M. A. Faria thanks FCT for the researcher contract through CEECINSTLA/00029/2022. The authors acknowledge the kind supply of insect species by CORIAL FOODS (SFP - Sustainable Food Products Lda, Esposende, Portugal).

References:

- [1] Orkusz, Nutrients, vol. 13, no. 4, 2021.
- [2] S. S. Teixeira, C. Villa, J. Costa, I. M. P. L. V. O. Ferreira, and I. Mafra, Foods, vol. 12, no. 10, 2023.
- [3] S. S. Teixeira, C. Villa, S. F. Sousa, J. Costa, I. M. P. L. V. O. Ferreira, and I. Mafra, Food Research International, vol. 169, 2023.
- [4] S. S. Teixeira, B. Carriço-Sá, C. Villa, J. Costa, I. Mafra, I. M. P. L. V. O. Ferreira, M. A. Faria, and T. G. Tavares, Foods, vol. 13, no. 21, 2024.

Challenges in food allergen quantification: DNA versus proteins

Joana Costa^{*}, Caterina Villa, Isabel Mafra

REQUIMTE/LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

^{*}*jbcosta@ff.up.pt*

Food allergies are a problem of public health, representing a current societal challenge. To protect the food-allergic consumers, it is urgent to exploit efficient analytical tools able to quantify food allergens. Currently, analytical methods can either target proteins or DNA, although there is still no consensus on the most adequate target, nor on the type of analytical technique [1]. This work intends to provide protein- and DNA-based methods with high efficiency for allergen detection and potential to be applicable in quality control laboratories or industrial context.

Different methodologies based on real-time PCR and ELISA technologies are presented as case studies to detect plant/animal allergenic foods (tree nuts/cow's milk), highlighting their advantages/drawbacks and applicability [2-7]. For DNA as a target analyte, novel single-tube nested real-time PCR approaches have been advanced to detect several tree nuts (hazelnut, almond, walnut, pistachio and cashew nut) in processed foods, targeting allergen-encoding genes. All systems provided high specificity (no cross-reactivity) and sensitivity, allowing quantifications between 5-50 mg/kg of the target nut in distinct matrices (cakes, biscuits, ice-creams). For protein as a target analyte, two indirect ELISA were developed targeting one cow's milk allergen each (β -lactoglobulin and caseins), with the latter presenting higher sensitivity (10 mg/kg), especially when analyzing extremely processed food matrices (e.g., autoclaved sausages).

In this context, the selection of the analytical method should be challenged by various parameters (sensitivity, specificity, target allergenic food, type of food matrix and degree of processing) and the purpose of the analysis. Herein, efficient allergen detection methods are proposed able to protect 90-99% of food allergic consumers, although its selection needs to be carefully considered on a case-by-case basis. At present, all these systems have been fully developed, validated and applied in our lab, providing useful tools for routine analysis and addressing the challenges of the food industry.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and through project HYPOALLERGEN (PTDC/BAA-AGR/4005/2021). C. Villa, J. Costa, and I. Mafra thank FCT for funding the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03583.CEECIND/CP1662/CT0012 and 2021.03670.CEECIND/CP1662/CT0011, respectively).

References:

- [1] T. Holzhauser, P. Johnson, J.P. Hindley, G. S. O'Connor, C.-H. Chan, J. Costa et al. Food and Chemical Toxicology, 145 (2020) 111709.
- [2] J. Costa, I. Mafra, T. Kuchta, M.B.P.P. Oliveira. Journal of Agricultural and Food Chemistry, 60(2012) 8103-8110.
- [3] J. Costa, M.B.P.P. Oliveira, I. Mafra. Food Research International, 51 (2013) 228-235.
- [4] J. Costa, M.B.P.P. Oliveira, I. Mafra. Food Research International, 54 (2013) 1722-1729.
- [5] J. Costa, I. Silva, C. Villa, I. Mafra. Journal of Food Composition and Analysis, 115 (2023) 105042.
- [6] J. Costa, C. Villa, L. Grazina, I. Mafra. Food Chemistry, 397 (2022) 133778.
- [7] C. Villa, M.B.M.V. Moura, J. Costa, I. Mafra. β -Lactoglobulin versus casein indirect ELISA for the detection of cow's milk allergens in raw and processed model meat products. Food Control, 135 (2022) 108818.

Can food processing and digestibility impact the allergenicity of shellfish and insect foods?

**Bruno Carriço Sá¹, Carla S.S. Teixeira, Caterina Villa¹, Eulália Mendes¹,
Isabel M.P.L.V.O. Ferreira¹, Isabel Mafra¹, Joana Costa^{1*}**

¹REQUIMTE-LAQV/Faculty of Pharmacy University of Porto, Porto, Portugal

* jbcosta@ff.up.pt

Shellfish allergies are concerning health conditions due to their high prevalence, persistence and severity of clinical symptoms. Recently, with the authorisation of 4-insect species (*Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus* and *Locusta migratoria*) for human consumption within the European Union (EU), the complexity of food allergen management has increased, since insects act as primary sensitisers and/or as cross-reactive species [1,2]. Therefore, this work intends to (1) optimise the extraction of allergens from the 4-EU regulated insects and (2) provide information on protein allergenicity of different species of shellfish (crustaceans/molluscs) and insects as affected by food processing and digestibility.

In the first case, 16 protein extraction protocols were tested for the 4-insect species, selecting a simple/fast extraction protocol (one-step incubation with 100 mM Tris-HCl+4% SDS, pH 7.6, buffer at 60°C/2h) as the most efficient in isolating insect allergens. Most of the insect allergens were IgE-reactive with sera from crustacean-allergic patients, showing their potential cross-reactivity with shellfish allergens [3]. In the second case, different food processing treatments (boiling, roasting, baking, frying, grilling) were performed in shellfish (shrimp, lobster, octopus, etc) and insect species. In general, food processing (thermal treatments) increased the allergenicity of shellfish/insects, especially in the case of tropomyosin (panallergen), which might be related to the structural alteration of certain allergens turning their epitopes more accessible to IgE-binding. Despite these results, there was a commercial flour of *A. diaperinus* that did not show allergenicity with sera from crustacean allergic patients, but whose industrial processing treatments were not disclosed. The digestibility of insects is currently being tested, but the digestibility of shellfish seems to decrease their protein allergenicity.

This work demonstrated the potential cross-reactivity of insects in shellfish allergic patients and the potentiality of processing to modulate crustaceans/molluscs and insect allergenicity towards the development of hypoallergenic foods formulations.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos and through project HYPOALLERGEN (PTDC/BAA-AGR/4005/2021). C. Villa, I. Mafra, and J. Costa thank FCT for funding the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03670.CEECIND/CP1662/CT0011 and 2021.03583.CEECIND/CP1662/CT0012, respectively). The authors acknowledge the kind supply of insect species by Corial Foods (SFP - Sustainable Food Products Lda, Esposende, Portugal)

References:

- [1] S. de Gier, K. Verhoeckx. *Molecular Immunology*, 100 (2018) 82-106.
- [2] J. Klueber, J. Costa, S. Randow, F. Codreanu-Morel, K. Verhoeckx, C. Bindslev-Jensen, M. Ollert, K. Hoffmann-Sommergruber, M. Morisset, T. Holzhauser, A. Kuehn. *Clinical & Experimental Allergy*, 50 (2020) 105-116.
- [3] B. Carriço-Sá, C.S.S. Teixeira, C. Villa, E. Mendes, I.M.P.L.V.O. Ferreira, I. Mafra, J. Costa. *Food Chemistry*, 468 (2025) 142453.

Nanoplate digital PCR for food quality and safety testing: application to botanical authentication of bacopa food supplements

Rita Biltres¹, Caterina Villa¹, Joana Costa¹, Isabel Mafra^{1,*}

¹REQUIMTE/LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

* isabel.mafra@ff.up.pt

Currently, awareness about the safety and quality of food supplements has been increasing. Plant food supplements (PFS), including plant extracts and botanical-based formulas, are among the most popular and widely consumed. However, despite their benefits, the lack of effective regulation increases the probability of contamination and mislabelling/adulteration, particularly the substitution with other botanicals. *Bacopa monnieri*, commonly known as bacopa or Brahmi, is a traditional medicine with several pharmacological properties, being used specifically as nerve tonic and nootropic booster [1] and, therefore, it is included in PFS. This work aimed at developing a new method for the detection and quantification of bacopa in PFS using nanoplate digital PCR (ndPCR). Recently, digital PCR has arisen as a newsworthy alternative to real-time PCR for plant species detection in processed and complex foods.

The proposed new ndPCR method exhibited high sensitivity, acceptable performance parameters and robustness, being applied to commercial samples of PFS. The transfer of real-time PCR to ndPCR was successfully accomplished. Compared to real-time PCR, the nanoplate digital PCR probe-based method was more sensitive, not being affected by matrix components.

The present work demonstrates the applicability of ndPCR for the detection of botanical species in complex and processed matrices for the first time, emphasizing its feasibility as a novel sensitive and specific tool to verify labelling compliance of PFS.

Acknowledgments: This work received financial support from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects “POIROT: novel methods and approaches for detecting the illegal addition of Pharmaceutical drugs and bOtanlcal adulteratiOn in planT food supplements” (PTDC/SAU-PUB/3803/2021 DOI 10.54499/PTDC/SAU-PUB/3803/2021) and UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020). C. Villa, J. Costa and I. Mafra thank FCT for funding through the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03583.CEECIND/CP1662/CT0012 DOI 10.54499/2021.03583.CEECIND/CP1662/CT0012 and 2021.03670.CEECIND/CP1662/CT0011 DOI 10.54499/2021.03670.CEECIND/CP1662/CT0011, respectively).

References:

- [1] G. Kuruvalli, et al.. Pharmacognosy Reviews, 17 (2023) 418-425.

Towards trending functional foods: combining spirulina with alternative protein sources

Rita Biltes¹, Carla Teixeira¹, Caterina Villa¹, Joana Costa¹, Isabel M. P. L. V. O. Ferreira¹, Isabel Mafra^{1,*}

¹LAQV/REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

* isabel.mafra@ff.up.pt

Spirulina (*Arthrospira platensis* and *Arthrospira maxima*) is a widely cultivated cyanobacteria, accounting for over 30% of global 'microalgal' biomass production [1]. It is recognised for its rich nutritional profile, containing 50 to 70 g/100 g of protein, polyunsaturated fatty acids (PUFA), essential vitamins (B1, B2, B3, B6, B9, B12, C, D, E), minerals (K, Ca, Fe, Mg, Zn) and antioxidants such as β -carotene and phycocyanins. Spirulina has been associated with several health beneficial properties, including the reduction of blood sugar levels, decreased blood pressure, reduced inflammation, among other bioactivities [2]. Consequently, spirulina is marketed as a valuable and strategic ingredient in the domain of functional food innovations. Spirulina-based products have been available in the European Union (EU) market since before 15 May 1997, with historical consumption records dating back to the 16th century in regions such as Mexico [3].

In response to the growing demand for nutrient-dense, sustainable protein sources, we have been developing four novel protein food formulations/products incorporating spirulina with alternative proteins to enhance both nutritional value and consumers' appeal. A survey of consumers was conducted to assess market viability, evaluating awareness, interest and acceptance of spirulinabased products, as well as other protein alternatives. Subsequently, a sensory evaluation was conducted to evaluate key attributes such as taste, texture, colour acceptance and overall consumers' perception.

The results obtained from this study are encouraging, demonstrating the feasibility of integrating spirulina into innovative food products, while providing valuable insights into consumer preferences and sensory acceptance. Having in mind the rising demand of sustainable and health-promoting foods, spirulina-based formulations present a promising opportunity for food innovation, industrial-scale production and commercialisation.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. C. Villa, I. Mafra, and J. Costa thank FCT for funding the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03670.CEECIND/CP1662/CT0011 and 2021.03583.CEECIND/CP1662/CT0012, respectively). The authors are grateful for the supply of Spirulina biomass from Tomar Natural (tomarnatural.pt/, Tomar, Portugal).

References:

- [1] T. Lafarga, J. M. Fernández-Sevilla, C. González-López, F. G. Acién-Fernández. Food Research International, 137 (2020) 109356.
- [2] N. K. Z. AlFadhly, N. Alhelfi, A. B. Altemimi, D. K. Verma, F. Cacciola, A. Narayanankutty. Molecules, 27 (2022) 5584.
- [3] J. D. Cruz, V. Vasconcelos. Foods, 13 (2024) 124.

Biosensors as pioneering solutions for monitoring food allergens and diagnosing food allergy

Catarina Dias^{1,2}, Patrícia Moreira^{1,2}, Caterina Villa², Isabel Mafra², Carlos M. Pereira¹, Joana Costa^{2*}, Renata Costa¹

¹ Centro de Investigação em Química da Universidade do Porto/Instituto de Ciências Moleculares (CIQUP-IMS), Faculdade de Ciências da Universidade do Porto, Porto, Portugal

² REQUIMTE-LAQV, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal

* jbcosta@ff.up.pt

Food allergy is a global health problem, whose prevalence seems to be increasing [1]. Presently, food allergen monitorization and allergy diagnosis is a challenging task, which highlights the unmet need for the advancement of highly sensitive and specific biosensors for allergen detection and allergy diagnosis. Biosensors have become increasingly important tools due to their simplicity, speed, ease of use, feasibility of automation and low cost. This work intends to present our latest biosensor advances for quantifying various food allergens (soybean/hazelnut) and total IgE in serum (allergy diagnosis).

For food allergen monitoring, several biosensors have been advanced targeting soybean and hazelnut allergens (Gly m Ti/Cor a 14, respectively) by distinct biorecognition platforms, namely molecular imprinting polymer (MIP), antibodies (IgG) and allergen-encoding genes (DNA) [2-6]. All systems enabled high specificity/sensitivity, allowing quantifying food allergens in the range of 0.1-10 mg/kg in matrix. Sensitivity levels depended on the biorecognition platforms, with immunosensor and MIP attaining the best limits of detection (0.1-1 mg/kg), regardless the target (soybean/hazelnut allergens) and the food matrix/processing (biscuits, hams, sausages) [3-6]. All biosensing systems were suitable for detecting traces of soybean/hazelnut in processed and complex foods, protecting 95-99 % of food-allergic patients.

For allergy diagnosis, an immunosensor targeting total IgE in the sera of food-allergic patients is currently being developed as a rapid and in situ alternative to the typical serological IgE determination performed at specialized laboratories/hospitals.

In this context, biosensors can be considered as useful strategies to quantify: (1) food allergens in situ (automated potential), aiding food industry to manage food allergens; and (2) IgE, based on the immunorecognition of specific molecular markers, thus aiding the clinical diagnosis of food allergy. All the systems are in place and ready for testing according to the need of food industry.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos, UIDB/00081/2020 (CIQUP), LA/P/0056/2020 (IMS) and through project HYPOALLERGEN (PTDC/BAA-AGR/4005/2021). C. Dias and P. Moreira thank FCT for PhD grants 2024.04195.BD and 2022.11382.BD, respectively. C. Villa, I. Mafra, and J. Costa thank FCT for funding the Individual Call to Scientific Employment Stimulus (2023.07644.CEECIND/CP2842/CT0011, 2021.03670.CEECIND/CP1662/CT0011 and 2021.03583.CEECIND/CP1662/CT0012, respectively). RC thanks FCT for funding through program DL 57/2016 – Norma transitória (SFRH/BPD/89752/2012).

References:

- [1] M.L.K. Tang, R.J. Mullins. Internal Medicine Journal, 47(2017) 256-261.
- [2] P. Moreira, J. Costa, C. Villa, I. Mafra, A.T.S.C. Brandão, C. Dias, A.F. Silva, C.M. Pereira, R. Costa. Analytica Chimica Acta, 1259 (2023) 341168.
- [3] R. Costa, J. Costa, P. Moreira, A.T.S.C. Brandão, I. Mafra, A.F. Silva, C.M. Pereira. Analytica Chimica Acta, 1191 (2022) 339310.
- [4] R. Costa, J. Costa, I. Sagastizábal, A.T.S.C. Brandão, P. Moreira, I. Mafra, A.F. Silva, C.M. Pereira. Food Chemistry, 361 (2021) 130122.
- [5] C. Dias, D. Fernandes, J. Costa, C. Villa, P. Moreira, A.T.S.C. Brandão, I. Mafra, A.F. Silva, C.M. Pereira, R. Costa. Analytica Chimica Acta, 1332 (2024) 343340.
- [6] C. Dias, J. Costa, I. Mafra, D. Fernandes, A.T.S.C. Brandão, A.F. Silva, C.M. Pereira, R. Costa. Talanta, 268 (2024) 125284.

Selection of aptamers to target enterotoxigenic *Escherichia coli* infections

Ana Maria Campos^{1,2,*}, Margarida Barros^{1,3}, Ricardo Oliveira^{1,4,5}, Carina Almeida^{1,4,5}

¹National Institute for Agricultural and Veterinary Research (INIAV), Oeiras, Portugal

²CEB- Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

³CECAV - Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

⁴LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto

⁵ ALiCE – Associate Laboratory in Chemical Engineering, Porto, Portugal

*anamaria.campos@iniav.pt

Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of enteric infections in swine, leading to significant economic losses in the swine industry [1]. Fimbriae, particularly F18-type, is a key virulence factor responsible for the initial adhesion of ETEC to intestinal epithelial cells, commonly associated with post-weaning diarrheal infections. Traditionally, this disease has been treated with antibiotics, but growing antimicrobial resistance has called for alternative approaches [2].

Aptamers, small single-stranded DNA or RNA oligonucleotides with specific 2D/3D structures, offer a promising strategy to block the initial adhesion of F18-ETEC. This work focuses on the *in vitro* selection of aptamers using an innovative cell-SELEX method targeting F18-ETEC and evaluating their affinity dissociation constant (K_d) to identify highly specific candidates.

Cell-SELEX begins with a random library of chemically synthesized oligonucleotides subjected to iterative steps of incubation with the target (F18+ ETEC), sorting, and amplification, culminating in a pool of sequences with high target affinity. The K_d was measured by incubating aptamers at varying concentrations with a constant bacterial cell count ($\approx 10^8$ CFU/mL).

The selected aptamers demonstrated low K_d values, that ranged from 67.61 nM [95% CI: 24.12–174.7] to 146.8 nM [95% CI: 54.44–405.6], reflecting strong binding affinities. Statistical analysis showed no significant differences between the selected aptamers for the target. Future work should focus on specificity assays with other bacterial species to determine the most suitable candidates for further development.

Acknowledgments: This work was financially supported by: Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and by FCT under the scope of the strategic funding of UIDB/04469/2020 unit (CEB). The authors are also grateful to FCT for the PhD Grants 2023.04664.BDANA and 2023.03705.BDANA.

References:

- [1] Fairbrother and Nadeau, "Colibacillosis," in *Diseases of swine*, 11th ed., K. J. S. Jeffrey J. Zimmerman, Locke A. Karriker, Alejandro Ramirez and J. Z. Gregory W. Stevenson, Eds. John Wiley & Sons, Inc, 2019, pp. 807–832.
- [2] V. García, M. Gambino, K. Pedersen, and S. Haugegaard, "F4- and F18-Positive Enterotoxigenic *Escherichia coli* Isolates from Diarrhea of Postweaning Pigs: Genomic Characterization," *Appl. Environ. Microbiol. - Genet. Mol. Biol.*, vol. 86, no. 23, 2020, doi: 10.1128/AEM.01913-20.

Insects as a sustainable alternative to animal protein: assessing digestibility, allergenicity and inflammatory potential

Lisete M. Silva^{1,*}, Inês E. Silva¹, Sara da Silva¹, Rafaela Fantatto², Marisa V. Santos³, Ricardo Dias⁴, Susana Soares¹, Victor Freitas¹

¹Faculty of Sciences of University of Porto, Porto, Portugal

²EntoGreen, Santarém, Portugal

³Thunder Foods, Santarém, Portugal ⁴LAQV-REQUIMTE, Porto, Portugal

* lisete.silva@fc.up.pt

Insect-derived proteins are gaining interest as an alternative to traditional animal proteins due to concerns over greenhouse gas emissions, land use, and water consumption in livestock production [1]. Insects offer high protein contents, a complete amino acid profile, and versatility for food applications. Although a few studies suggest their digestibility may match or exceed that of traditional proteins, it varies by species and processing methods [2, 3]. Thus, assessing their digestibility, allergenicity and inflammatory potential is crucial for evaluating their suitability as food ingredients.

In this work, dehydrated *Hermetia illucens* (also known as Black Soldier Fly, BSF) and *Tenebrio molitor* (TM) larvae, as well as the defatted insect meals, and protein extracts (PE) obtained after a meticulous protein extraction optimization and characterization, were subjected to *in vitro* gastrointestinal digestions, following the INFOGEST protocol [4]. The samples were desalted and analyzed by nanoLC-MS/MS. The allergenicity potential of the resulting peptides were predicted *in silico* using the AllerCatPro 2.0 web server [5]. The pro-inflammatory response of BSF and TM meals and their corresponding PE was assessed using a transwell-based human intestinal epithelial cell model consisting of differentiated Caco-2 and HT-29-MTX co-cultures [6].

For BSF samples, 1076 peptides (0.6-3.6 kDa) were identified, with 0.2 % in the PE, and 0.9 % in the larvae and meal showing strong allergenicity potential. For TM, 1329 peptides (0.6-3.9 kDa) were identified, with 0.9 % in the PE, 3.9 % in the meal and 2.6% in the larvae predicted as allergenic. Despite these results, further tests are needed to confirm the allergenicity potential of these insect-based ingredients. None of the insect samples induced a significant pro-inflammatory response under the tested conditions – monitored through IL-8 release -, supporting their potential as sustainable food ingredients.

Acknowledgments: This work received funding from the Recovery and Resilience Plan (PRR) within the scope of the Mobilizing Agenda for Business Innovation; project reference C644917393- 00000032: “InsectERA – A ERA da indústria dos insetos”.

References:

- [1] K.J. Hawkey, C. Lopes-Viso et al. Annual Review of Animal Biosciences, 9 (2021) 333.
- [2] L. Yi, C.M.M. Lakemond, et al. Food Chemistry, 141(4) (2013) 3341.
- [3] Z. Ma, M. Mondor et al. Food & Function, 14(18) (2023) 8129.
- [4] Brodkorb, L. Egger et al. Nature Protocols, 14(4) (2019).
- [5] M.N. Nguyen, N.L. Krutz, et al. Nucleic Acids Research, 50(W1) (2022) W36.
- [6] S. da Silva, R. Pérez-Gregorio et al. Food Research International, 173 (2023), 113317.

Evaluating children's preferences for sustainable menus in school canteen´

Solinho J.^{1,2,3*}, Mata, F². Pereira-Pinto, R.^{1,2}, Pinheiro R.^{1,2}

¹School of Technology and Management, Polytechnic Institute of Viana do Castelo, Avenida Atlântico 4900-348 Viana do Castelo, Viana do Castelo, Portugal

²Centre for Research and Development in Agrifood Systems and Sustainability (CISAS) of the Polytechnic Institute of Viana do Castelo, Avenida Atlântico 4900-348 Viana do Castelo, Viana do Castelo, Portugal

³EDIUS – International PhD School of the USC, University of Santiago de Compostela, 15705 Santiago de Compostela, Spain

* jsolinho@ipvc.pt

Fish, as a key component of a balanced diet, providing essential nutrients that support children's growth and cognitive development [1]. Nutrition in early life is increasingly considered an important factor influencing future health, meaning that early experiences with various flavours and textures play a key role in shaping long-term eating habits [2]. School programmes have the potential to promote the influence of peers and incorporate repeated exposure to healthy foods, which is known to increase acceptance over time [3].

The study assessed the preferences of 192 primary school children aged 6–10 years for two sustainable typical Portuguese dishes: “*Massada de Peixe*” - pasta cooked with *Atlantic bonito* and “*Arroz de Peixe*” - rice cooked with *Atlantic bonito*. Participants rated their appreciation of each dish on a five-point hedonic scale ranging from 1 (dislike extremely) to 5 (like extremely). The goal was to identify the preferred dish for potential inclusion in school canteen menus and to examine how age and appreciation levels influence preferences.

Using logistic regression analysis, the study examined the relationship between dish preference (pasta or rice) as the dependent variable and “Age” and “Appreciation” as independent variables. Results indicated that older children, who had a high appreciation of fish-based meals, significantly preferred rice with Atlantic bonito. On the other hand, younger children, particularly six-year-olds, preferred pasta with Atlantic bonito, showing a lower overall appreciation for fish dishes.

These results underscore the importance of designing sustainable school menus that align with children's taste preferences to improve acceptance and reduce food waste.

The study emphasizes the value of incorporating sensory assessments and consumer feedback into menu development processes.

Furthermore, it underlines the role of knowledge and education in fostering acceptance of sustainable meals, suggesting that initiatives aimed at increasing awareness of fish consumption and sustainability can improve the appeal of such dishes among young customers.

Acknowledgements: The authors thank the Blue Project, Bioeconomy, People, Sustainability, Health (PT-INNOVATION-0105). Iceland Liechtenstein Norway EEA grants. Blue Growth Programme. Call2 – Business, Development, Innovation and SMEs.

References:

- [1] Hibbeln, J. R., Davis, J. M., Steer, C., Emmett, P., Rogers, I., Williams, C., & Golding, J. (2007). Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *The Lancet*, 369(9561), 578-585. [https://doi.org/https://doi.org/10.1016/S0140-6736\(07\)60277-3](https://doi.org/https://doi.org/10.1016/S0140-6736(07)60277-3)
- [2] De Cosmi, V., Scaglioni, S., & Agostoni, C. (2017). Early Taste Experiences and Later Food Choices. *Nutrients*, 9(2). <https://doi.org/10.3390/nu9020107>
- [3] Blanchette, L., & Brug, J. (2005). Determinants of fruit and vegetable consumption among 6–12-year-old children and effective interventions to increase consumption. *Journal of Human Nutrition and Dietetics*, 18(6), 431-443. <https://doi.org/https://doi.org/10.1111/j.1365-277X.2005.00648.x>

Hemp seeds and their role in promoting health and sustainability

Andreia Saragoça^{1,*}, Ana Cordeiro^{1,2}

¹ Biosciences School of Elvas, Polytechnic Institute of Portalegre, Elvas, Portugal

² MED—Mediterranean Institute for Agriculture, Environment and Development, & CHANGE—Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Polo da Mitra, Ap. 94, Évora, 7006-554, Portugal

*18954@ipportalegre.pt

Hemp (*Cannabis sativa* L.) is a multifunctional, sustainable crop with a positive environmental impact, belonging to the Cannabaceae family. It is cultivated for the production of textiles, food, paper, biofuels, medicines, and hygiene products [1, 2]. The seeds of *Cannabis sativa* L., particularly low-THC varieties, are recognized for their nutritional value. They contain high-quality proteins, such as edestin and albumin, which provide a complete profile of essential amino acids, comparable to protein sources like soy and whey [3–5]. Hemp seeds contain 25–35% oil with significant nutritional properties, rich in polyunsaturated fatty acids (PUFAs), including linoleic acid and α -linolenic acid. This characteristic makes them a promising option for applications in both the food and cosmetic industries, standing out as a versatile and beneficial ingredient [4, 6, 7]. In addition to being rich in lipids, hemp seeds have a diverse nutritional composition, including proteins (20–30%), carbohydrates (10–15%), insoluble fiber, vitamins, and a variety of essential minerals such as phosphorus, potassium, magnesium, sulfur, calcium, iron, and zinc [4, 8]. Equally relevant is the fact that hemp seeds are valued for their bioactive compounds, such as phenolics, tocopherols, lignan amides, and peptides, which confer antioxidant and anti-inflammatory activities [9–11]. These benefits make hemp a promising functional food for improving public health, reducing the risk of chronic diseases without significant interventions [12, 13]. The seeds of *Cannabis sativa* L. stand out for their nutritional value, containing high-quality proteins, beneficial lipids, and bioactive compounds with antioxidant and anti-inflammatory properties. Their benefits for human and animal health, along with their potential for food and cosmetic applications, reinforce their role as a promising functional food in promoting well-being and preventing diseases.

References:

- [1] J. Fike, Critical Reviews in Plant Sciences. 35 (2016) 406–424.
- [2] V. Kitryté, D. Bagdonaitė, P. Rimantas Venskutonis, Food Chemistry. 267 (2018) 420–429.
- [3] R.E. Aluko, in Sustainable Protein Sources, S. R. Nadathur, J. P.D. Wanasundara, L. Scanlin, Elsevier, 2017 121–132.
- [4] J.C. Callaway, Euphytica. 140 (2004) 65–72.
- [5] C.-H. Tang, Z. Ten, X.-S. Wang, X.-Q. Yang, J. Agric. Food Chem. 54 (2006) 8945–8950.
- [6] G. Crescente, S. Piccolella, A. Esposito, M. Scognamiglio, A. Fiorentino, S. Pacifico, Phytochem Rev. 17 (2018) 733–749.
- [7] M. Pojić, A. Mišan, M. Sakač, T. Dapčević Hadnađev, B. Šarić, I. Milovanović, M. Hadnađev, J. Agric. Food Chem. 62 (2014) 12436–12442.
- [8] J.D. House, J. Neufeld, G. Leson, J. Agric. Food Chem. 58 (2010) 11801–11807.
- [9] A.T. Girgih, A. Alashi, R. He, S. Malomo, R.E. Aluko, Eur J Nutr. 53 (2014) 1237–1246.
- [10] X. Yan, J. Tang, C. Dos Santos Passos, A. Nurisso, C.A. Simões-Pires, M. Ji, H. Lou, P. Fan, J. Agric. Food Chem. 63 (2015) 10611–10619.
- [11] N.M. Rodriguez-Martin, S. Montserrat-de La Paz, R. Toscano, E. Grao-Cruces, A. Villanueva, J.-Pedroche, F. Millan, M. C. Millan-Linares, Biomolecules. 10 (2020) 803.
- [12] H.P.V. Rupasinghe, A. Davis, S.K. Kumar, B. Murray, V.D. Zheljazkov, Molecules. 25 (2020) 4078.
- [13] P. Cerino, C. Buonerba, G. Cannazza, J. D'Auria, E. Ottoni, A. Fulgione, A. Di Stasio, B. Pierri, A. Gallo, Cannabis and Cannabinoid Research. 6 (2021) 19–27.

Impact of cellulose nanocrystals as a new food ingredient on microbiota health

Ana Isabel Bourbon^{1,*}, Mafalda Neto¹, Catarina Gonçalves¹, Lorenzo Pastrana¹

¹ INL - International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n 4715-330, Braga, Portugal

* ana.bourbon@inl.int

Cellulose nanocrystals are revolutionizing the food industry, opening new possibilities both in the development of active packaging and in innovative formulations for creamy and pasty foods. This study explores the behavior of cellulose nanocrystals during in vitro digestion, using both a conventional reactor and a microfluidic (on-chip) system, based on the Infogest protocol. During in vitro digestion it was observed the impact of gastro and intestinal conditions could affect the agglomeration of cellulose nanocrystals and impact their behaviour in contact with intestinal cells. Furthermore, the impact of nanocrystals on human microbiota health was assessed through the evaluation of short fatty acids profile and advanced PCR techniques, and the effects of fermentation products on intestinal epithelial integrity was analyzed using cellular models (Caco-2 and HT29-MTX).

In order to evaluate the safety of CNC in food applications, cytotoxicity and genotoxicity tests were performed using human intestinal cell lines. Caco-2 cells were exposed to different CNC concentrations for 24 h, and then cell viability and DNA damage were assessed by resazurin conversion assay and alkaline comet assay, respectively.

Acknowledgments: Ana Isabel Bourbon acknowledges funding by FCT, through the individual scientific employment program contract (2020.03447.CEECIND).

Sweet-tasting oligosaccharides as a strategy for functional sugar reduction in foods

Pedro A. R. Fernandes^{1,*}, Bruna L. Antunes¹, Abigail González², Clarisse Nobre^{2,3}, Vitor D. Alves⁴, Adriana Silva⁴, Mário Cristóvão⁵, Cláudia Nunes⁶, Elisabete Coelho¹, Manuel A. Coimbra¹

¹ LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal;

² CEB—Centre of Biological Engineering, University of Minho, Braga, Portugal

³ LABBELS —Associate Laboratory, Braga, Portugal

⁴ Frulact, 4475-150 Gemunde – Maia, Portugal;

⁵ Centro de Apoio Tecnológico Agroalimentar (CATAA), 6000-459, Castelo Branco, Portugal

⁶ CICECO – Aveiro Institute of Materials, Department of Materials and Ceramic Engineering, University of Aveiro, Aveiro, Portugal

* pedroantonio@ua.pt

Industries have been making efforts to replace sugar in foods by using sweet tasting molecules. However, most of the currently available solutions provide off-taste profiles, alongside with the loss of texture perceived by the consumer as negative [1]. Besides sucrose, fructose and glucose, many oligosaccharides can provide sweetness and texture to foods, but without significant caloric contribution. These include fructooligosaccharides (FOS), yacon carbohydrates that are unable to be metabolized by human digestive enzymes given their backbone structure composed of a sucrose unit extended by 1)→Fruf-(β2→ chains [2]. Therefore, FOS comprise carbohydrates that might build up a perfect carbohydrate solution for sugar reduction in foods by providing sweetness, texture, and promoting gut health. Based on this hypothesis, this work studied the functional properties of FOS-rich syrups obtained from yacon, an inulin-rich tuberous root characteristic of the Andean region [3]. To achieve this, yacon juice was extracted and concentrated until obtaining a syrup of 80 °Brix. The obtained syrup was then used as a sugar replacer in strawberry formulations. The syrup was also studied concerning the stability of FOS upon simulated gastrointestinal digestion, including gut fermentation.

The yacon syrups contained different FOS structures, including 1-kestose, nystose and 1,1,1-kestopentaose. When replacing 50% of sugar in strawberry formulations, FOS increased the firmness of the formulation from 30 g to 85 g, reflecting the strong water retention capacity of the syrups. When submitted to gastrointestinal simulation, it was observed that the syrups were able to promote the growth of *Bifidobacterium* and foster the production of short chain fatty acids. The capacity of FOS to maintain sweetness and texture of foods with reduced sugar content, while contributing to the maintenance of gut health, showcase the potential of sweet-tasting oligosaccharides as a premium sugar reduction strategy.

Acknowledgments: The authors acknowledge CICECO (UIDB/50011/2020 (DOI 10.54499/UIDB/50011/2020), UIDP/50011/2020 (DOI 10.54499/UIDP/50011/2020) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020)), financed by national funds through the FCT/MCTES (PIDDAC). The authors also acknowledge to the project “cLabel+: Alimentos inovadores “clean label” naturais, nutritivos e orientados para o consumidor”, number 46080, POCI-01-0247-FEDER-046080 and LISBOA-01-0247-FEDER-046080. Thanks to YaconPortugal for providing samples. A. G. acknowledges FCT for the PhD grant SFRH/BD/06268/2021.

Funding: This work was financially supported through LAQV/REQUIMTE (LA/P/0008/2020 DOI <https://doi.org/10.54499/LA/P/0008/2020> , UIDB/50006/2020 DOI <https://doi.org/10.54499/UIDB/50006/2020> , UIDP/50006/2020 DOI <https://doi.org/10.54499/UIDP/50006/2020>).

References:

- [1] Gomes, A. I. Bourbon, A. R. Peixoto, A. S. Silva, A. Tasso, C. Almeida, C. Nobre, C. Nunes, C. Sánchez, D. A. Gonçalves, D. Castelo-Branco, D. Figueira, E. Coelho, J. Gonçalves, J. A. Teixeira, L. M. P. Castro, M. A. Coimbra, M. Pintado, M. A. P. R. Cerqueira, P. Fuciños, P. Teixeira, P. A. R. Fernandes, V. D. Alves, in Food structure engineering and design for improved nutrition, health and well-being, M. A. P. R. Cerqueira, L. M. P. Castro (Eds.), Elsevier Inc., 2022.
- [2] T. M. Pereira, D. M. Pereira, A. C. Hiramatsu, E. Y. Ventura, M. B. Bolini, H. M. André, LWT, 150 (2021) 112054.
- [3] P. A. R. Fernandes, B. L. Antunes, J. Liu, S. S. Ferreira, F. Fernandes, V. D. Alves, A. Silva, C. Nunes, E. Coelho, M. A. Coimbra, Appl. Sci., 14 (2024) 894.

Determining the LC₅₀ of hydro-ethanolic extract of *Eriobotrya japonica* leaves from Azores region on zebrafish embryos for toxicological insights

Jorge Barros^{1,2*}, Luís Félix¹, Ana Abraão¹, Irene Gouveinhas¹, Daniel Granato³, Ana Barros^{1*}

¹Centre for Research and Technology of Agro-Environmental and Biological Sciences, CITAB, Inov4Agro, University of Trás-os-Montes and Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal.

²Department of Agricultural sciences, Higher Polytechnic Institute of Bengo, Website: www.ispbengo.ao – facebook.com/ispbengo | B. Caboxa | Dande | Bengo, Angola.

³Bioactivity & Applications Lab, Department of Biological Sciences, Faculty of Science and Engineering, School of Natural Sciences, University of Limerick, V94 T9PX Limerick, Ireland.

* jorsongomes2000@gmail.com (J.G.L.B.); abarros@utad.pt (A.N.B.)

Eriobotrya japonica, commonly called loquat, is a fruit tree from the Chinese subtropical region. Its diverse organs have been used for thousands of years in traditional medicine, namely leaves. In fact, the leaves of this plant contain phytoconstituents, such as phenolics, with therapeutic properties, making them capable of neutralizing inflammation, diabetes, cancer, bacterial infection, aging, pain, allergies, and other health problems. However, studies investigating *in vivo* metabolism, bioavailability, and structure-activity relationships, as well as the potential toxicity of these extracts in animal or cellular models are poorly explored [1-3].

This study aimed to find out the LC₅₀ of hydro-ethanolic extract from *Eriobotrya japonica* leaves from Azores region on zebrafish embryos as a part of toxicological analysis.

Briefly, two hours after fertilization, the animals were collected and then subjected to different concentrations of hydro-ethanolic extract of *Eriobotrya japonica* leaves (0, 25, 50, 100, 200, and 400 mg/L) in E3 buffer for 96 hours with replacement of the solution and recording of mortality at 24h, 48h, 72h, and 96h [4].

After 96h, the LC₅₀ was calculated at the end of the experiment and a value of 189.8 mg/L was obtained. According to OECD guidelines (OECD, 2013), LC₅₀ values ranging from 10 to 100 mg/L are harmful, 1 to 10 mg/L are toxic, and any value below 1 mg/L is highly toxic [5]. Thus, the LC₅₀ value of the leaf extract under study was higher than the value suggested by the guideline, indicating that it can be considered safe. Additional studies with LC₁₀ and other values closer to LC₅₀ will be carried out to ensure the extract is safe.

Acknowledgments: Jorge Barros acknowledges the financial support provided by the Project PRT/BD/152099/2021. This work is also supported by National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UI/04033 and LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>). We would also like to express our gratitude to Biokairós for generously providing the samples for analysis.

References:

- [1] M. Ruaa. Iraqi Journal of Pharmaceutical Sciences. 30 (2021), 41-55. P-ISSN: 1683 - 3597, E-ISSN: 2521 - 3512) 10.31351/vol30iss1p41-55.
- [2] X. Zhu, L. Wang, T. Zhao, Q. Jiang, Q. Journal of Ethnopharmacology. 298 (2022) 115566.
- [3] T. Khouya, M. Ramchoun, H. Elbouny, H. Abdelbassat, E. Bouhlali, T. Eimad Ch. Alem, Chakib. Journal of Ethnopharmacology. 296 (2022) 115473. 10.1016/j.jep.2022.115473.
- [4] J. R. Meyers, Current Protocols Essential Laboratory Techniques, 19 (2018) doi: 10.1002/cpet.
- [5] Josje H.E. Arts, H. Muijsers, D. Jonker, J. Sandt, P. Bos, V. Feron. OECD guidelines in relation to REACH and scientific developments, Journal of Experimental and Toxicologic Pathology, 60, 2–3 (2008), 125-133. ISSN 0940-2993, doi:10.1016/j.etp.2008.01.011.

Giving a second life to winery by-products: development of crackers formulation as a health promoter

Carlos Eduardo Moreira Pinto^{1*}, Sofia Mariana Saavedra Silva¹, José Miguel Lemos¹, Estevan Versolato Carballo¹, Luís Ferreira², Ana Novo Barros²

¹ Oenology Students in UTAD (University of Trás-os-Montes and Alto Douro) *
carlosmpinto10@gmail.com

² Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB)/Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro), University of Trás-os-Montes and Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal

*abarros@utad.pt

The winery industry generates a significant amount of by-products such as grape pomace, seeds, stems, and lees [1]. These by-products have been traditionally used as animal feed or for composting. However, due to their high content of bioactive compounds, they have gained interest in the food industry for their potential use as functional ingredients in the development of new foods with nutraceutical properties.

The bioactive compounds present in this industry's by-products include phenolic compounds, that have been associated with several health benefits such as antioxidant, anti-inflammatory, anti-cancer, and cardio protective effects. In fact, several *in vitro*, *in vivo*, and clinical studies have demonstrated that food products enriched with wine industry by-products possess even cardio protective properties [2]

Therefore, the inclusion of these by-products in food production has many health benefits and represents an excellent opportunity to recycle waste streams and add value to the wine industry. In this context, the aim of this study was to evaluate the effects of the incorporation of wine lees from the Douro region in the formulation of crackers, regarding the taste, appearance, and functional properties. The development of a cracker formulation, using winery by-products as a health promotor, offers a second life to something that is usually considered waste [3]

Based on previous studies, it is expected that the incorporation of lees into bread formulations will increase the antioxidant capacity of the product. Moreover, this study will also assess the taste, appearance, and functional properties of the crackers to determine its overall quality [4].

References:

- [1] Barba, F.J.; Zhu, Z.; Koubaa, M.; Sant'Ana, A.S.; Orlie, V. Green Alternative Methods for the Extraction of Antioxidant Bioactive
- [2] Compounds from Winery Wastes and By-Products: A Review. Trends Food Sci. Technol. 2016, 49, 96–109, doi:10.1016/j.tifs.2016.01.006.
- [3] Teixeira, A.; Baenas, N.; Dominguez-Perles, R.; Barros, A.; Rosa, E.; Moreno, D.A.; Garcia-Viguera, C. Natural Bioactive Compounds from Winery By-Products as Health Promoters: A Review. Int. J. Mol. Sci. 2014, 15, 15638–15678, doi:10.3390/ijms150915638.
- [4] Jara-Palacios, M.J., Wine Lees as a Source of Antioxidant Compounds. Antioxidants. 2019, 8, 45, doi:10.3390/antiox8020045
- [5] Ferrer-Gallego R., Silva P., The Wine Industry By-Products: Applications for Food Industry and
- [6] Health Benefits. Antioxidants, 2022, 11, 2025, doi.org/10.3390/antiox11102025

Strategies to increase prebiotic activity and neurotrophic potential through dietary fibre modulation

Soraia P. Silva¹, Abigail Gonzalez², Dalila Roupar², Andreia F. Salvador^{2,3}, Rita Bastos⁴, Mariana Mota¹, Maria H. Luís¹, Sónia S. Ferreira¹, Maria J. Alegria⁵, Clarisse Nobre², Manuel A. Coimbra¹, and Elisabete Coelho^{1,*}

¹ LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Aveiro, Portugal

² CEB—Centre of Biological Engineering, University of Minho, Braga, Portugal

³ LABBELS –Associate Laboratory, Braga, Portugal

⁴ Colab4Food – Collaborative Laboratory, INIAV - Vairão, Vila do Conde, Portugal

⁵ SUMOL COMPAL Marcas S.A, R. Dr. António João Eusébio, n.º24, 2790-179, Carnaxide, Portugal

* ecoelho@ua.pt

Soluble polysaccharides and oligosaccharides are prebiotic compounds that promote the growth of beneficial gut bacteria and contribute to improved health. Industrial juice production results in juices with moderate amounts of soluble dietary fibre because most polysaccharides that constitute the dietary fibre are retained in the pulp. Incorporation of glucanases and pectinases into the juice production process can therefore enrich the juice with soluble fibre and possible prebiotic effects. Apple cloudy juice was only composed of 4 % of fibre (dry basis), mainly pectic polysaccharides. Polysaccharides (mostly insoluble fibre rich in cellulose and pectic polysaccharides) are retained in pulpe (41-37 %) after the industrial juice processing. Hydrolysis of apple pomace with endo- and exo-cellulase released a high amount of material (63.9 %), composed of monosaccharides and a variety of cellulose and xyloglucan oligosaccharides. Hydrolysis with pectin lyase, polygalacturonase, and pectin methylesterase released 57.7 % material composed of pectic polysaccharides and derived oligosaccharides, showing a wide variety of oligosaccharide structures. Then, they were fermented with fecal inocula, increasing the concentration of short-chain fatty acids (SCFAs). When compared with the non-treated enzymatically pomace was observed a larger increase in propionate followed by acetate and butyrate, and high amounts of γ -aminobutyric acid (GABA) and 5-hydroxytryptophane (5-HTP) during fermentation. Enzymatic treatment with pectinases improved the intestinal microbiota with the promotion of psychobiotics, and the production of neurotransmitters.

In lignified by-product materials, more drastic strategies need to be used to recover polysaccharides and oligosaccharides with prebiotic activity. Pine nut skin, a by-product obtained during pine nut processing, is a source of insoluble dietary fiber¹. Subcritical water extraction allowed to obtain an extract rich in phenolic compounds and mono- and oligosaccharides, and an extract rich in pectic polysaccharides and xyloglucans². The fermentation of mono- and oligosaccharides and phenolic compounds did not cause a significant microbial shift upon fermentation. Nonetheless, it stimulated significantly ($p < 0.05$) the growth of the prebiotic *Bifidobacterium adolescentis* and the short-chain fatty acids production, specifically acetate and propionate. Pectic polysaccharides and xyloglucans had an impact on the microbiota composition similar ($p < 0.05$) to fructooligosaccharides used as positive control, also increasing the abundance of *B. adolescentis*. The core microbiome upon these polysaccharides' fermentation was composed of multiple butyrate producers, which lead to an increase ($p < 0.05$) of butyrate concentration. Besides, it was observed a microbial utilization selectivity towards xyloglucans in detriment to rhamnose-containing pectic polysaccharides and type II arabinogalactans. The highest concentration of GABA was attained at 24 h of fermentation, with a decrease at 48 h. This decrease can be related to the utilization of GABA to produce succinic acid. Pine nut skin can be considered a source of soluble oligo- and polysaccharides with prebiotic effect, promoting the growth of psychobiotics which may help reduce depression, anxiety, and modulate mood.

Acknowledgments: This work was funded by VIIAFOOD-1.2.2. FFV Fruit Fractioning and Valorization from PRR (Programa de Recuperação e Resiliência). Authors acknowledge the FCT financial support through LAQV/REQUIMTE (10.54499/UIDB/50006/2020, 10.54499/UIDP/50006/2020, and 10.54499/LA/P/0008/2020), and through UIDB/04469/2020 unit and LA/P/0029/2020 within the PT2020 Partnership Agreement, thank FCT/MCTES for PhD grants (SFRH/BD/136471/2018, SFRH/BD/06268/2021 and SFRH/DB/139884/2018), and E.Coelho research contracts (DL 57/2016/CP1482/CT0038, 2023.08855.CEECIND).

References:

- [1] S. P. Silva, P. Ferreira-Santos, G. R. Lopes, S. F. Reis, A. González, C. Nobre, V. Freitas, M. A. Coimbra, E. Coelho, Carbohydr. Polym. Technol. Appl., 7 (2024) 100508.
- [2] S. P. Silva, F. Coreta-Gomes, M. A. Coimbra, E. Coelho, LWT, 197 (2024) 115934.

Understanding the motives behind the consumption of cow's milk and its plant-based substitutes in two contrasting European markets

Sofia Sousa^{1,2}, Diogo Aguiar², Jorge C. Oliveira³, Luís M. Cunha^{1,2*}

¹ GreenUPorto/INOV4Agro, Faculty of Sciences, University of Porto, 4485-646 Vila do Conde, Portugal

² DGAOT, Faculty of Sciences, University of Porto, 4485-646 Vila do Conde, Portugal

³ School of Engineering and Architecture, University College Cork, College Road, T12 YN60 Cork, Ireland

* lmcunha@fc.up.pt

In Europe, cow's milk faces competition from plant-based milk substitutes (PBMS), which are gaining popularity, especially among young consumers¹⁻⁶. We studied the determinants of cow's milk and PBMS consumption in two distinct market contexts: Ireland (major world milk producer and consumer, especially pasteurised⁷) and Portugal (less dominant market, mostly UHT⁸). Young adults (n=645, 18-35 years) completed a self-administered online questionnaire disseminated through the University of Porto and University College Cork academic communities. The motives behind the consumption and non-consumption of cow's milk and PBMS were assessed using four 10-item 7-point anchored scales. Exploratory and multigroup confirmatory factor analyses were used to identify factors within each scale and confirm measurement invariance across countries. Binary logistic regression was performed to quantify associations between cow's milk/PBMS consumption and sociodemographic and dietary characteristics. Among cow's milk consumers (57.2% of all participants), the Portuguese preferred UHT milk (93.8%) and the Irish pasteurised milk (95.8%). Among PBMS consumers (54.4%), almond "milk" was the most popular (Portugal: 67.3%; Ireland: 74.1%). PBMS consumption (and cow's milk non-consumption) were generally related to health and environmental sustainability motivations, while taste was the main reason for preferring cow's milk over PBMS. Gender, education, level of trust in different sources of diet-related information, dietary patterns and food restrictions showed significant associations with cow's milk and PBMS consumption, although differently among Portuguese and Irish consumers.

This study highlights the importance of optimising specific product characteristics of cow's milk and PBMS, with a special focus on the sensory profiles and the environmental and health impacts of these product categories, to approach young consumers' expectations. Solid scientific information on these topics must be produced and communicated to consumers to enable informed food choices.

Acknowledgements: The authors acknowledge support from the strategic project UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável, funded by national funds via FCT (Foundation for Science and Technology - Portuguese Ministry of Science, Technology and Higher Education). The authors acknowledge Diogo Aguiar's internship at the University College Cork, with the support of the Erasmus+ programme of the European Union.

References:

- [1] United States Department of Agriculture, Dairy and Products Semi-annual - European Union. 2023, United States Department of Agriculture Foreign Agricultural Service: Warsaw.
- [2] The Good Food Institute Europe, Europe Plant-Based Foods Retail Market Report (2020-2022). 2022, GFI Europe.
- [3] Onwezen, M.C., et al., A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. *Appetite*, 2021. 159: p. 105058. Must be formatted according to the examples presented below
- [4] Ruzgys, S. and G.J. Pickering, Perceptions of a sustainable diet among young adults. *Environmental Education Research*, 2024: p. 1-17.
- [5] Moss, R., et al., An investigation into consumer perception and attitudes towards plant-based alternatives to milk. *Food Research International*, 2022. 159: p. 111648.
- [6] Martínez-Padilla, E., et al., Perceptions toward Plant-Based Milk Alternatives among Young Adult Consumers and Non-Consumers in Denmark: An Exploratory Study. *Foods*, 2023. 12(2): p. 385.
- [7] Agriculture and Food Development Authority, The End of the Quota Era: A History of the Irish Dairy Sector and Its Future Prospects, ed. T. Donnellan, T. Hennessy, and F. Thorne. 2015, Ireland.
- [8] European Union, Analysis of the Portuguese dairy sector to 2030 - Dairy4Future Work Package 4: dairy sector analysis. 2021.

Impact of previous exposure to fraud on Portuguese consumer perceptions and attitudes towards food fraud

Isabel Sousa^{1,2,3†}, Maria João Costa^{1,2†}, Ana Pinto de Moura^{1,4}, José António Teixeira³ and Luís Miguel Cunha^{1,2*}

¹GreenUPorto- Sustainable Agrifood Production Research Centre/INOV4Agro, University of Porto, Vila do Conde, Portugal

²DGAOT, Faculty of Sciences, University of Porto, Porto, Portugal

³CEB-Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal

⁴DCeT, Universidade Aberta, Porto Portugal

[†]Equal contribution

*lmcunha@fc.up.pt

Consumers are becoming increasingly sceptical and cautious about the foods they buy and consume [1,2]. The attitudes and behaviours of Portuguese consumers about food chain fraud remain little understood [3,4]. This study evaluates consumers' perceptions, attitudes, and reported behaviours toward food fraud. A total of 368 participants, recruited through digital devices, fully answered a self-reported questionnaire in LimeSurvey. Descriptive statistics, χ^2 test, the Mann-Whitney test, or the Wilcoxon test were used, where appropriate. Factor analysis was applied to reduce the main dimensions of the questionnaire, and binary logistics regression was also computed to evaluate the determinants of carrying out self-protective behaviours to minimise the risk of exposure to food fraud.

Consumers are generally aware of the motivations of food fraud perpetrators, namely the motivation to obtain greater profits. They also reveal great concerns about health in the context of food fraud, emphasising measures to prevent it; they also present low levels of trust in products, systems, and authorities. This is emphasised by consumers previously exposed to food fraud. Meat, fish, poultry, and dietary supplements represent the categories most vulnerable to food fraud, and e-commerce, traditional markets, and foodservice channels are the most susceptible stages in the food supply chain. Older consumers and women appear to understand food fraud better. Although consumers intend to report food fraud when facing a food fraud situation, only a few have been reported by official channels. Self-protective behaviours to minimise the risk of exposure to food fraud include the type of store, brand, packaging, product origin, and search for food fraud information. Consumer's perception of fraudsters' motivations, health concerns, measures to prevent food fraud, level of trust in products, systems and authorities, age, and previous exposure to fraud seem to be involved in adopting these behaviours.

Acknowledgments: Acknowledgments: Authors Costa, Moura, and Cunha acknowledge support from the strategic project UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável, funded by national funds via FCT (Foundation for Science and Technology - Portuguese Ministry of Science, Technology and Higher Education).

References:

- [1] H. Kendall, B. Clark, C. Rhymer, S. Kuznesof, J. Hajslova, M. Tomaniova, P. Brereton, L. Frewer, L, Trends in Food Science and Technology, 94 (2019)
- [2] J. Barnett, F. Begen, S. Howes, A. Regan, A. McConnon, A. Marcu, S. Rowntree, W. Verbeke, Food Control, 59 (2016)
- [3] M.J. Costa, I. Sousa, A. Pinto Moura, J.A. Teixeira, L.M. Cunha, Journal of Food Protection, 87(2024)7.
- [4] M.J. Moreira, J. García-Díez, J.M.M.M. de Almeida, C. Saraiva, Foods, 10 (2021) 5

Exploring Phenolic Content and Antioxidant Properties of Enzyme-assisted Extracts from Red Seaweed *Porphyra dioica*

Paulo Nova^{1,2*}, Ana R. Costa-Pinto^{2,3}, Ana Maria Gomes¹

¹Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal;

²i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;

³IPATIMUP – Instituto de Patologia Molecular e Imunologia da Universidade do Porto, Porto, Portugal
*pnova@ucp.pt

Among the many seaweed species, *Porphyra dioica* (red, *Rhodophyta*) is highlighted for its important nutritional profile, in particular, its richness in compounds with antioxidant capacity. This work investigates the antioxidant capacity and total phenolic content of enzyme-assisted extracts (employing either a protease -alcalase®, or one of the two cell wall degrading carbohydrases - cellulase® and Viscozyme® - a multi-enzyme complex of carbohydrases) from the seaweed *Porphyra dioica* (PD) obtained from land-based fully controlled cultivation systems under the Integrated Multi-Trophic Aquaculture (IMTA) sustainable concept and provided by ALGApplus® (Aveiro, Portugal). Total phenolic content was determined by the Folin-Ciocalteu method. Antioxidant capacity was measured by three different complementary *in vitro* assays: ABTS total antioxidant capacity, DPPH free radical, and oxygen radical absorbance capacity (ORAC)). Results are presented in Table 1.

Highest total phenolic content, total antioxidant capacity and oxygen radical absorbance capacity were obtained for the extraction with alcalase (PDa), whereas the cellulase extraction (PDC) registered the highest results for DPPH free radicals. Viscozyme extraction showed no additional value on phenolic content and antioxidant capacity in comparison with cellulase extraction. This study presents promising applications of enzyme-assisted extraction for obtaining antioxidant-rich extracts from the red seaweed *P. dioica*.

Table 1. Total phenolic content and antioxidant capacity of *Porphyra dioica* enzyme-assisted extracts obtained with alcalase, cellulase, or viscozyme.

	Total phenolic content mg Gallic acid Equiv/g dry extract	DPPH mg Trolox Equiv/g dry extract	ABTS mg Trolox Equiv/g dry extract	ORAC mg Trolox Equiv/g dry extract
PD alcalase	14.73 ± 0.23 ^a	0.2525 ± 0.013 ^a	20.04 ± 0.44 ^a	130.01 ± 8.99 ^a
PD cellulase	9.37 ± 0.49 ^b	0.3028 ± 0.026 ^a	12.58 ± 0.36 ^b	104.33 ± 5.40 ^b
PD viscozyme	9.54 ± 0.42 ^b	0.2960 ± 0.018 ^a	11.88 ± 0.61 ^b	111.39 ± 3.86 ^b

^{a-b}, in a column: different letters indicate significant differences ($p < 0.05$) between enzyme-assisted procedures.

Acknowledgments: This work was developed under the framework of Paulo Nova Individual FCT PhD Research Grant (ref. SFRH/BD/05747/2020) financed by Fundação para a Ciência e Tecnologia (FCT) and project Agenda VIIAFOOD – Platform for Valorisation, Industrialization and Commercial Innovation for the Agri-Food Industry”, financed through the Program for Recovery and Resilience (PRR). The authors would like to acknowledge ALGApplus® (Aveiro, Portugal) for providing the powdered seaweed samples, and the scientific collaboration under the FCT project UIDB/50016/2020.

References:

- [1] Nova P, Pimenta-martins A, Silva JL, Machado A, Gomes AM, Freitas AC. Health benefits and bioavailability of marine resources components that contribute to health – what's new? Crit Rev Food Sci Nutr [Internet]. 2020;0(0):1–13. Available from: Nova P, Martins AP, Teixeira C, Abreu H, Silva JG, Silva AM, et al. Foods with microalgae and seaweeds fostering consumers health: a review on scientific and market innovations. J Appl Phycol. 2020;14.
- [2] Suresh V, Senthilkumar N, Thangam R, Rajkumar M, Anbazhagan C, Rengasamy R, et al. Separation, purification and preliminary characterization of sulfated polysaccharides from
- [3] Sargassum plagiophyllum and its in vitro anticancer and antioxidant activity. Process Biochem [Internet]. 2013;48(2):364–73. Available from: <http://dx.doi.org/10.1016/j.procbio.2012.12.014>
- [4] Gião MS, González-Sanjósé ML, Rivero-Pérez MD, Pereira CI, Pintado ME, Malcata FX. Infusions of Portuguese medicinal plants: Dependence of final antioxidant capacity and phenol content on extraction features. J Sci Food Agric. 2007;87(14):2638–47.
- [5] Coscueta ER, Reis CA, Pintado M. Phenylethyl isothiocyanate extracted from watercress byproducts with aqueous micellar systems: Development and optimisation. Antioxidants. 2020;9(8):1–11.
- [6] Rodrigues D, Freitas AC, Queirós R, Rocha-Santos TAP, Saraiva JA, Gomes AMP, et al. Bioactive Polysaccharides Extracts from Sargassum muticum by High Hydrostatic Pressure. J Food Process Preserv. 2017;41(1):1–12.
- [7] Rodrigues D, Sousa S, Silva A, Amorim M, Pereira L, Rocha-Santos TAP, et al. Impact of enzyme- and ultrasound-assisted extraction methods on biological properties of red, brown, and green seaweeds from the Central West Coast of Portugal. J Agric Food Chem. 2015;63(12):3177–88.
- [8] Nova P, Cunha SA, Costa-Pinto AR, Gomes AM. Chemical and Antioxidant Properties of Solvent and Enzyme-Assisted Extracts of Fucus vesiculosus and Porphyra dioica. Mar Drugs. 2024;22(7):319.

Germinated quinoa (*Chenopodium quinoa*): Effects on mineral composition, antioxidant capacity, and technofunctional properties

Silvia Melissa García-Torres^{1,*}; José António Teixeira²; Christian René Encina-Zelada³; Cristina Luisa Miranda Silva¹; Ana Maria Gomes¹

¹Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia - Porto, Portugal

² Centre of Biological Engineering, School of Engineering, University of Minho - Braga, Portugal

³Departamento de Tecnología de Alimentos, Facultad de Industrias Alimentarias, Universidad Nacional Agraria La Molina - Lima, Perú

* s-smgtorres@ucp.pt

The transformation of food systems is key to combating malnutrition and food insecurity, focusing on sustainability, the preservation of biodiversity, and equitable access to healthy diets. In this context, the adoption of friendly processing techniques, such as the germination of grains like quinoa, could play a fundamental role. Although germination has been shown to improve various aspects of quinoa's nutritional, functional, technofunctional, and sensory profiles, its effects on these factors can be complex. Quinoa varieties Rosada de Huancayo (RH) and Pasankalla (PK) from Perú, were washed, disinfected, and germinated at different combinations (T1-T9) of temperature (15 °C, 20 °C, 25 °C) and time (24h, 48h, 72h). After the process, the grains were dried and prepared for analysis. Mineral determination was performed using ICP-OES spectroscopy, while antioxidant capacity was evaluated with DPPH, ABTS, and ORAC assays. Additionally, techno-functional properties were analyzed. Germination increased calcium and sodium levels in both quinoa varieties ($p<0.05$). In Pasankalla, T9 (25 °C, 72h) maintained molybdenum levels. However, a decrease in the concentration of other key minerals, such as iron and magnesium, was observed in germinated quinoa compared to non-germinated quinoa ($p<0.05$). The ABTS method showed a significant increase in antioxidant capacity in Rosada de Huancayo ($p<0.05$). Regarding the technofunctional properties, Pasankalla exhibited better swelling power, oil absorption capacity, and emulsification activity (EA) ($p<0.05$). Water absorption capacity (WAC) in T8 was similar to non-germinated quinoa ($p>0.05$). In Rosada de Huancayo, T9 and T7 showed significant differences in WAC and EA compared to the control. Germination time/temperature combinations affected differently the mineral profile, antioxidant capacity, and technofunctional properties of quinoa grains. While improvements were observed in calcium and sodium levels, and some functional properties, other minerals and antioxidant capacity decreased. Germination shows promise for modifying quinoa's properties but requires careful evaluation of process conditions based on intended use.

Acknowledgments: This work was developed under the framework of project Agenda VIIAFOOD – Platform for Valorisation, Industrialization and Commercial Innovation for the Agri-Food Industry”, financed through the Program for Recovery and Resilience (PRR). The authors would like to acknowledge the scientific collaboration under the FCT project UIDB/50016/2020.

References:

- [1] The State of Food Security and Nutrition in the World 2023. Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum. FAO; IFAD; UNICEF; WFP; WHO. Rome, FAO, 2023.
- [2] Fardet, A. Journal of Cereal Science. 60 (2014) 3.
- [3] Darwish, A. M. G., Al- Jumayi, H. A. O., & Elhendy, H. A. Cereal Chemistry, 98 (2021) 2.
- [4] Thakur, P., Kumar, K., Ahmed, N., Chauhan, D., Eain Hyder Rizvi, Q. U., Jan, S., Singh, T. P., & Dhaliwal, H. S. Current Research in Food Science, 4 (2021).

Development of 3D-printed structured food models using anthocyanin-rich double emulsion gels

Liandra Gracher-Teixeira^{1,2,3}, Ana Carolina Lima^{1,2,3}, Elsa Ramalhosa¹, Arantzazu Santamaria-Echart¹, Madalena M. Dias^{2,3}, M. Filomena Barreiro^{1,*}

¹CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

²LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Porto, Portugal

³ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

* *barreiro@ipb.pt*

The food industry must offer healthful, appealing products. Natural colorants like anthocyanins from black carrots are gaining attention as safer alternatives to synthetic ones [1]. However, their instability under different conditions poses challenges [2]. 3D printing is a promising tool for creating personalized food, particularly for those with specific dietary needs like dysphagia [3]. Double emulsion systems provide a potential solution by safeguarding sensitive compounds while attaining desirable properties for 3D printing [4]. This research examines anthocyanin-rich double emulsion gels for 3D printing, emphasizing bioactive compound protection and suitable textural properties for dietary uses. The experimental approach followed the methodology described in a previous study with modifications [5]. Briefly, the water-in-oil-in-water ($W_1/O/W_2$) double emulsions (DE) were prepared using black carrot extract (8 wt.%) in the inner aqueous phase, corn oil with PGPR (5 wt.%) as the oil phase, and an external aqueous phase containing gelatine at various concentrations (4%, 6.5%, and 10 wt.%), gum Arabic (6.5 wt.%), and Tween 80 (3 wt.%). All formulations' encapsulation efficiency reached 99.97%, and anthocyanin bioaccessibility was significantly improved. The DE_6.5% formulation (i.e., the DE with 6.5% gelatine) showed the highest bioaccessibility (36.10±0.022%), representing a 3.64-time increase compared to the free form (9.91±0.02%). Rheological studies demonstrated that increasing gelatine content promotes the formation of a denser polymer network, improving the structural stability and printability of the emulsion gels. The 6.5% and 10 wt.% gelatine formulations produced 3D-printed structures with good shape retention and stability. Texture analysis showed the highest hardness in DE_10% (135.58±5.38 g), suitable for dysphagia diets. The printed structures also conform with the International Dysphagia Diet Standardization Initiative (IDDSI) criteria. This study shows that combining double emulsion gels with 3D printing creates a promising method for developing functional foods that protect bioactive compounds and meet dietary needs, especially for individuals with swallowing difficulties.

Acknowledgments: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); LSRE-LCM UIDB/50020/2020 (DOI:10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI:10.54499/UIDP/50020/2020); ALiCE LA/P/0045/2020 (DOI:10.54499/LA/P/0045/2020), and individual research grants 2020.08803.BD of Liandra Gracher-Teixeira, and 2021.05378.BD of A. C. Lima. National funding by FCT, P.I., through the institutional scientific employment program contract of A. Santamaria-Echart.

References:

- [1] Chikane, P. Rakshe, J. Kumbhar, A. Gadge, S. More, International Journal of Scientific Research in Science and Technology, 9, (2022) 547–550.
- [2] Enaru, G. Dreţcanu, T.D. Pop, A. Stănilă, Z. Diaconeasa, Antioxidants, 10 (2021) 1967.
- [3] Y. Xie, Q. Liu, W. Zhang, F. Yang, K. Zhao, X. Dong, S. Prakash, Y. Yuan, Foods 12 (2023) 1842.
- [4] Aniya, Y. Cao, C. Liu, S. Lu, Y. Fujii, J. Jin, Q. Xia, Foods 12 (2022) 151.
- [5] L. Gracher-Teixeira, S.C.S. Pituco, G. Colucci, A. Santamaria-Echart, A.M. Peres, M.M. Dias, M.F. Barreiro, Foods 13 (2024).

Chemical characterization of *Agaricus bisporus* by-products for development of functional ingredients

Maria Inês Arranca^{1,2,*}, Filipe Coreta-Gomes^{1,3}, Elisabete Coelho¹, Rita Bastos²

¹ LAQV-REQUIMTE, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal

² Associação Colab4Food - Laboratório Colaborativo para a Inovação da Indústria Agroalimentar, Vairão, Portugal

³ Coimbra Chemistry Center-Institute of Molecular Sciences (CQC-IMS), Department of Chemistry, University of Coimbra, 3004-535 Coimbra, Portugal

* inesarranca@ua.pt

Mushroom consumption in Portugal have been increased 10-15% per year, mostly due to their organoleptic properties, and following current market trends such as the consumption of healthy, vegan or high protein food ¹. The national production is 5.3 ktons per year, from which 5 to 10% are by-products, which are currently used as fertilizer or feed, with low added commercial value². However, these by-products are a rich source of polysaccharides, like β -glucans with potential hypocholesterolemic effect^{3,4} as well as proteins.

In this work, extraction and characterization of different fractions containing polysaccharides and proteins from *Agaricus bisporus* by-products was addressed. Ethanolic and aqueous extracts of *Agaricus bisporus* by-products resulted in low yield of soluble (13%) and high yield of insoluble (39%) polysaccharides. The fractions are rich in sugars (37-60%) with glucose as a major component (44-73%mol). The final residue (WIR) (53% carbohydrates; 69%mol glucose; 19%mol glucosamine) yielded 39% of initial mushroom contained an insoluble glucan with similar quantities of (β 1 $\rightarrow$ 4)-Glc (30%) and (β 1 $\rightarrow$ 6)-glucan (23%). Its insolubility could be due to the presence of (1 $\rightarrow$ 4)-GlcNAc (11%) and GlcN (1%), resulting in chitin-glucan complex, and to the presence of 28% of protein.

The amount of β -glucans, chitin and protein may be promising for development of functional ingredients which can be used on the development of hypocholesterolemic food ingredients or nutritional and organoleptic claims, such as protein content and umami taste, respectively, which will contribute for the valorisation of mushroom by-products.

Acknowledgments: Maria Inês Arranca thanks to the FCT for her grant ref. 2024.03558.BDANA. Elisabete Coelho thanks Portuguese national funds (OE), through FCT, I.P., in the scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19 (CDL-CTTRI-88-ARH/2018 - REF. 049-88-ARH/2018).

Funding: The authors also acknowledge the funding through LAQV/REQUIMTE (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020, and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) and FCT/MCTES for the financial support of MTS/BRB/0056/2020.

References:

- [1] Imarc. (2024). *Mushroom Market Size*. Imarc.
- [2] Observador. (2024). *Empresa investe três milhões e aumenta produção de cogumelos para 6,4 toneladas em 2026* – Observador.
- [3] Inca-Torres, R., Aguilera-Velázquez, J. R., Urbina-Salazar, A. del R., Carbonero-Aguilar, P., Moreno Navarro, I., & Bautista Palomas, J. (2024). Enzymatic Preparation of Mushroom By-product Protein Hydrolysates (Mb-PPHs). *Waste and Biomass Valorization*, 15(3), 1509–1518.
- [4] Silva, I. M. V., Machado, F., Moreno, M. J., Nunes, C., Coimbra, M. A., & Coreta-Gomes, F. (2021). Polysaccharide structures and their hypocholesterolemic potential. *Molecules*, 26(4559).

Coffee brew can aid on fight against cholesterol?

Fernanda Machado^{1*}, Manuel A. Coimbra², Filipe Coreta-Gomes^{1,2,*}

¹LAQV-REQUIMTE, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal

² Coimbra Chemistry Centre - Institute of Molecular Sciences (CQC-IMS), University of Coimbra, 3004-535 Coimbra, Portugal

* *filipecoreta@ua.pt*

Coffee brew is well prevalent in human diet with several recognized health benefits [1,2]. These bioactive properties have been related with the chemical diversity which composes the coffee beverage, although structure-function relationships are still not disclosed. One of the compounds most prevalent in coffee brew are the soluble fibres, composed by arabinogalactans and galactomannans polysaccharides, and melanoidins, which may impact on cholesterol metabolism by several mechanisms [3].

The effect of chemically characterized coffee and polysaccharides rich fractions on cholesterol bioaccessibility was accessed using an *in vitro* intestinal model. Coffee as well as the arabinogalactans and galactomannans polysaccharides rich fractions were shown to decrease cholesterol solubility, due to their capacity to sequester bile salt. [4,5] Moreover, coffee soluble fibres, namely arabinogalactans and melanoidins rich fractions were also evaluated regarding the outcome of their fermentability using an *in vitro* colonic fermentation model. [6] Both fractions decreased the acetate: propionate ratio, which is indicative of a potential HMG-CoA reductase inhibition. Melanoidin rich fraction also show to decrease the conversion of primary to secondary bile salts. As secondary bile salts are more prone to emulsify cholesterol, their decrease will lead to a decline on cholesterol bioaccessibility and bioavailability. To investigate coffee and melanoidins effect on cholesterol metabolism *in vivo*, experiments using mice (C57BL/6) subjected to a high-fat diet (HFD) were performed, showing that coffee and melanoidins treatment led to higher levels of plasma total cholesterol and increase HDL-cholesterol levels. In addition, coffee samples decreased HFD-induced body weight gain and lipid accumulation, and improved insulin resistance.

This study demonstrates that coffee can have an impact on cholesterol metabolism, suggesting potential for developing functional food ingredients from coffee extracts to fight cardiovascular diseases, which is one the leading causes of death worldwide.

Acknowledgments: FCT/MCTES, LAQV-REQUIMTE (10.54499/LA/P/0008/2020, 10.54499/UIDP/50006/2020, 10.54499/UIDB/50006/2020) and the project PTDC/QUI-OUT/29373/2017 (OE, FEDER, POCI, PT2020); Machado acknowledges FCT PhD fellowship (2020.06768.BD); UC-NMR facility (FEDER, European Regional Development Fund, COMPETE Program) and FCT grants (RECI/QEQ-QFI/0168/2012, CENTRO-07-CT62-FEDER-002012); RNRMN and CCC (UID/QUI/00313/2019).

References:

- [1] F. Machado, M. A. Coimbra, M. D. del Castillo, and F. Coreta-Gomes, *Crit. Rev. Food Sci. Nutr.* **1** (2023).
- [2] M. F. Karagöz, E. Koçyiğit, T. Koçak, A. Özturan Şirin, M. A. Icer, D. Ağagündüz, and F. Coreta-Gomes, *Compr. Rev. Food Sci. Food Saf.* **23**, (2024).
- [3] I. Silva, F., Machado, M., Coimbra, *Molecules* **26**, 4559 (2021).
- [4] F. M. Coreta-Gomes, G. R. Lopes, C. P. Passos, I. M. Vaz, F. Machado, C. F. G. C. Geraldés, M. J. Moreno, L. Nyström, and M. A. Coimbra, *Nutrients* **12**, 437 (2020).
- [5] C. L. Pires, I. M. V. Silva, M. A. Coimbra, M. J. Moreno, and F. Coreta-Gomes, *Foods* **11**, 2935 (2022).
- [6] F. Machado, I. Gómez-Domínguez, R. Hurtado-Ribeira, D. Martin, M. A. Coimbra, M. D. del Castillo, and F. Coreta-Gomes, *Int. J. Biol. Macromol.* **275**, 133740 (2024).

Culinary Plant-Based Decoctions: A promising approach for combatting neurodegenerative disorders

Clara Grosso^{1,*}, Rosário Martins^{2,3}, Cristina Delerue-Matos¹

¹REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

² Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 400, 4200-072 Porto, Portugal

³ Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto (CIIMAR/CIMAR), Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

* claragrosso@graq.isep.ipp.pt

This study aimed to assess the effectiveness of nutritional therapy in preventing or treating neurodegenerative disorders. To achieve this, decoctions from 5 culinary plants - turmeric (*Curcuma longa* L.), rosemary (*Salvia rosmarinus* Spenn.), lemon basil (*Ocimum basilicum* L. var. *citriodorum*), spearmint (*Mentha spicata* L.) and winter savory (*Satureja montana* L.) - were evaluated for their ability to combat reactive oxygen species (ROS), reactive nitrogen species (RNS), and enzymes associated with Alzheimer's disease. The total phenolic content ranged from 43.2 mg gallic acid equivalent (GAE)/g extract in turmeric to 327.3 mg GAE/g extract in rosemary. While lemon basil, spearmint, rosemary and winter savory decoctions displayed significant superoxide anion radical scavenging activity (IC_{50} values between 46.8 and 62.0 $\mu\text{g/mL}$), winter savory was the least active of this group against nitric oxide radical ($IC_{50}=314.5 \mu\text{g/mL}$). Rosemary extract was the most active inhibitor of cholinesterases, exhibiting $IC_{50}=1068.4 \mu\text{g/mL}$ for acetylcholinesterase and $IC_{50}=858.8 \mu\text{g/mL}$ for butyrylcholinesterase. Turmeric decoction, with the lowest phenolic content, showed the least promising results among the tested extracts. This study demonstrates the potential of culinary plant decoctions as nutritional therapies for neurodegenerative disorders. Rosemary exhibited the most promising results, showing high activity in scavenging free radicals and inhibiting cholinesterases, suggesting its potential to be included in a functional food or food supplement.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Clara Grosso is thankful for her contract (2020.03436.CEECIND/CP1596/CT0008, DOI 10.54499/2020.03436.CEECIND/CP1596/CT0008) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.

Fatty acid profile and antioxidant activity of andiroba (*Carapa guianensis* Aubl.) seed oil

Matilde Rodrigues¹, Clara Batista^{1,2}, José Pinela^{1,3,*}

¹ CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

² Instituto Federal da Paraíba, Campus São Gonçalo, 58807-610 Sousa, Paraíba, Brasil

³ National Institute for Agricultural and Veterinary Research (INIAV), I.P., Rua dos Lágidos, Lugar da Madalena, 4485-655 Vairão, Vila do Conde, Portugal

* jose.pinela@iniav.pt

Andiroba oil is extracted from the seeds of *Carapa guianensis* Aubl. (Meliaceae), a tree native to the tropical regions of Central and South America, particularly the Amazon rainforest. Known for its medicinal and cosmetic properties, andiroba oil has been traditionally used by indigenous communities for centuries. Rich in bioactive compounds, it offers a wide range of therapeutic applications [1]. The oil promotes wound healing by enhancing fibroblast activity and collagen synthesis, while its high content of essential fatty acids helps restore the skin's natural barrier, accelerating the healing of wounds, burns, and ulcers. These properties make it a valuable ingredient in dermatological applications. In the cosmetic industry, andiroba oil is valued for its moisturizing and soothing effects on the skin. It can be found in formulations of creams, lotions, and hair care products, where it acts as an emollient, improving skin texture and hair manageability. Additionally, its anti-aging potential, attributed to its antioxidant properties, helps protect the skin from oxidative damage caused by environmental factors such as ultraviolet radiation [2]. Despite its extensive potential, andiroba oil remains little explored by both industry and the scientific community. This study aimed to characterize the fatty acid profile and evaluate the antioxidant activity of andiroba oil.

The fatty acid profile was analyzed using gas chromatography with flame ionization detection (GC-FID) after fatty acid derivatization with methanol/sulfuric acid/toluene (2:1:1, v/v/v). The antioxidant potential was determined *in vitro* through DPPH free radical scavenging activity and reducing power assay, using trolox as a positive control. The results revealed that the lipid fraction of andiroba oil is predominantly composed of oleic, palmitic, and linoleic acids. Additionally, it demonstrated high antioxidant activity in both DPPH free radical scavenging and reducing power assays. This research enhances the understanding of andiroba oil's composition and bioactive potential, opening new avenues for its application in different sectors. However, further studies are needed to fully explore its bioactive and pharmacological potential and ensure its safe application in the industrial sector.

Acknowledgments: The authors are grateful to the Foundation for Science and Technology (FCT) for financial support through national funds FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (10.54499/UIDB/00690/2020) and UIDP/00690/2020 (10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (10.54499/LA/P/0007/2020); and national funding by FCT, through the individual research grant (2024.04986.BD) of M. Rodrigues.

References:

- [1] Leal, et al., Ind Crops Prod, 202 (2023) 117004.
- [2] Silva, et al., Colloids Surf. A: Physicochem. Eng. Asp., 702 (2024) 134990.

Swine Industry: Validated PCR-based method for detecting enterotoxigenic *Escherichia coli*

Margarida Barros^{1,2*}, Joana Castro^{1,3}, Ricardo Oliveira^{1,5,6}, Ana Maria Campos^{1,3}, Daniela Araújo^{1,3,4}, Sónia Silva^{1,3,4}, Gonçalo Nieto Almeida¹, Divanildo Outor-Monteiro², Carina Almeida^{1,3,5,6}

¹ National Institute for Agrarian and Veterinarian Research (INIAV), Rua dos Lagidos, 4485-655 Vila do Conde, Portugal

² Veterinary and Animal Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

³ Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

⁴ LABBELS—Associate Laboratory, Braga/Guimarães, 4710-057 Braga, Portugal

⁵ LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁶ ALICE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

* margarida.barros@iniav.pt

Enterotoxigenic *E. coli* (ETEC) infections lead to significant costs for the swine industry worldwide [1]. Bacterial fimbriae (F4/F5/F6/F18) allow the adhesion of the bacteria to epithelial cells, and when both the immunological systems and the gut microbiota are poorly developed, ETEC colonizes and produces one or more enterotoxins (LT/STa/STb) that can have local and systemic effects [3,4]. Therefore, it is of prime importance to monitor and characterize ETEC in the swine industry to develop mitigation strategies.

In this study, we aimed to isolate and characterize ETEC strains from faecal porcine samples. Therefore, it was developed a PCR-based method to detect their major virulence factors (toxins/fimbriae). This procedure was divided into two procedures, firstly the collected faecal samples were enriched and then pre-screened for the presence of genetic determinants of toxins (ST/LT/Stx2) by real-time PCR (qPCR). Secondly, the positive enrichments were plated in Tryptone Bile X-glucuronide (TBX) agar and incubated at 37 °C for 24 hours. Fifty characteristic *E. coli* colonies were then extensively screened for the presence of toxins (STa/STb/LT/Stx2e) and fimbriae (F4/F5/F6/F18/F41) by multiplex-PCR. The development of this PCR-based method was done using ETEC controls harbouring the above-mentioned toxins/fimbriae.

For qPCR, the primer efficiency, exclusivity/inclusivity, and limit of detection (LOD) in ETEC isolates and artificially inoculated real samples were evaluated to validate the methodology. For the multiplex-PCR, exclusivity/inclusivity assays were performed for both sets (toxins/fimbriae). Therefore, our results showed that the qPCR method could detect concentrations ranging from 2×10^{-5} to 2×10^{-3} ng.µL⁻¹, depending on the target gene, demonstrating high sensitivity and specificity. In addition, the multiplex PCR technique was successfully validated using ETEC control strains. In conclusion, this method has great potential for routine use in the rapid detection of ETEC strains in livestock, given its robust performance and reliability.

Acknowledgments: This work was financially supported by: Project PTDC/CVT-CVT/4620/2021, funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; LA/P/0045/2020 (ALICE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and by FCT under the scope of the strategic funding of UIDB/04469/2020 unit (CEB).

References:

- [1] J. M. Fairbrother and É. Nadeau, "Colibacillosis," in *Diseases of Swine*, 11th ed., J. J. Zimmerman, L. A. Karriker, A. Ramirez, K. J. Schwartz, G. W. Stevenson, and J. Zhang, Eds., John Wiley & Son, 2019, ch. 52, pp. 807–834.
- [2] Luppi, "Swine enteric colibacillosis: Diagnosis, therapy and antimicrobial resistance," Aug. 08, 2017, *BioMed Central Ltd.* doi: 10.1186/s40813-017-0063-4.
- [3] M. M. Barros et al., "Swine Colibacillosis: Global Epidemiologic and Antimicrobial Scenario," Apr. 01, 2023, MDPI. doi: 10.3390/antibiotics12040682.
- [4] J. Castro et al., "Swine enteric colibacillosis: Current treatment avenues and future directions," pp. 1–15, Oct. 2022, doi: 10.3389/fvets.2022.981207.

Replacing inorganic phosphate in clean-label cooked hams

Cristina Roseiro^{1,2*}, Helena Gonçalves¹, Luís Canhoto¹, Irene Cristina Antunes^{1,3}, Carlos Santos¹

¹ Instituto Nacional de Investigação Agrária e Veterinária, I.P. Unidade de Tecnologia e Inovação, Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

² GeoBioTec, Nova School of Science and Technology, Caparica, Portugal

³ Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, Universidade de Lisboa, Pólo Universitário Alto da Ajuda, 1300-477 Lisboa, Portugal.

* *cristina.roseiro@iniav.pt*

Traditional cooked hams processed with low brine injection levels are muscle foods highly consumed around the world because they show nice appearance and palatability, they are almost fat free and are very interesting nutritionally. However, the conventional technological process also makes use of negative impact additives in consumers due to potential health concerns, mainly the addition of nitrite and inorganic phosphates (1,2). The aim of this study was to investigate the technological and sensory impact of replacing phosphate with natural polysaccharides for the processing of whole muscle pork cooked hams with low brine extension level, contributing to the development of clean-label meat products.

Forty formulations were prepared by replacing phosphate with carrageenan, fructo-oligosaccharide fibers (citrus and inulin) and rice/corn starches. The formulations with carrageenan and citrus fiber presented higher L* values compared to samples with phosphate (control) ($P < 0.05$), showing that their utilization could favor lighter colored products due to the opaque color properties of their gels. a* values of all phosphate substitutes were lower than control ($P < 0.05$) but no significant differences were obtained in b* values. Cook losses varied between 7.4-14.6%, presenting the formulations with phosphate lower values than all the other formulations ($P < 0.05$). The formulations with phosphate, rice and corn starch presented higher hardness (69.4N, 67.6N, 64.1N, respectively) than carrageenan and citrus fiber (55.2N and 52.7N, respectively). The formulations with carrageenan and citrus fiber presented similar values to the control for the tenderness, slice cohesion and color homogeneity evaluated sensorially.

The present study is a new approach for use healthier ingredients with similar functionality to phosphate in cooked meat products. The results indicated that the use of carrageenan and citrus fiber as alternative ingredients to phosphates may be a good strategy for the production of clean-label cooked meat products.

References:

- [1] M. Petracci, M. Bianchi, S. Mudalal, C. Cavani. Functional ingredients for poultry meat products. Trends in Food Science & Technology, 33 (2013) 27-39.
- [2] V. C. Resconi, D. F. Keenan, M. Barahona, L. Guerrero, J. P. Kerry, R M. Hamill. Rice starch and fructo-oligosaccharides as substitutes for phosphate and dextrose in whole muscle cooked hams: Sensory analysis and consumer preferences. LWT - Food Science and Technology, 66 (2016) 284-292.

Fate of (poly)phenols from radish microgreens grown under different lights: Digestion and gut microbiota catabolism

Marta Silva^{1*}, José Fernando Rinaldi de Alvarenga², Lorena Sánchez-Martínez^{2,3}, Letizia Bresciani², Olga Viegas⁴, Miguel A. Faria¹, Isabel M.P.L.V.O. Ferreira^{*1}

¹ LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

² Human Nutrition Unit, Department of Food and Drugs, University of Parma, Parma, Italy
³ University of Murcia, Murcia, Spain

⁴ LAQV/REQUIMTE, Faculty of Nutrition and Food Sciences, University of Porto, Porto, Portugal

* Isabel.ferreira@ff.up.pt

Brassicaceae microgreens are emerging functional foods rich in (poly)phenols (1) being ideal to indoor cultivation where light is artificially supplied and can be tailored to optimize composition (1). However, their fate upon ingestion remains largely unknown, limiting the understanding of their true impact on health. Most (poly)phenols present low absorption in the small intestine, reaching the colon for further catabolism (2). Gut microbiota modifies these compounds, impacting their bioaccessibility and potential health effects (2). Therefore, this study investigated the *in vitro* digestion and colonic fermentation of two radish microgreens (*Raphanus sativus*) cultivars, Daikon and Red Rambo, grown under different light conditions in vertical farming, exploring how cultivar and light influence (poly)phenol catabolism. Gastrointestinal digestion was simulated using the INFOGEST *in-vitro* static protocol. *In vitro* batch colonic fermentation was performed according to validated protocols (Bresciani et al. (3), Dall'Asta et al. (4), Di Pede et al. (5)). (Poly)phenols and respective catabolites were analysed by UHPLC-ESI-QqQ-MS/MS. After digestion most (poly)phenols remained in the residual fraction reaching the colon, where microbial fermentation produced more bioavailable catabolites. Cultivar differences generally outweighed the effects of light treatments. Red Rambo retained twice the (poly)phenols of Daikon, influencing fermentation outcomes. Cinnamic acid derivatives, particularly sinapoyl malate and sinapine, dominated both cultivars, while flavonoids and anthocyanins, in Red Rambo, contributed with further complexity. Fermentation of these compounds produced important amounts of phenylpropanoic acids including dihydrocaffeic and dihydroferulic acids, and other low molecular weight catabolites. Other important microbiota-derived catabolites included sinapic, ferulic, *p*-coumaric, dihydroferulic, dihydrocaffeic, and 4-hydroxyphenylpropanoic acids. Proposed catabolic pathways for most (poly)phenols are outlined, with the pathway for the most abundant (poly)phenol, sinapoyl malate, being outlined for the first time. These relevant findings lay the foundation for future *in vivo* studies to delve deeper into compound absorption and bioactivity upon consumption of radish microgreens.

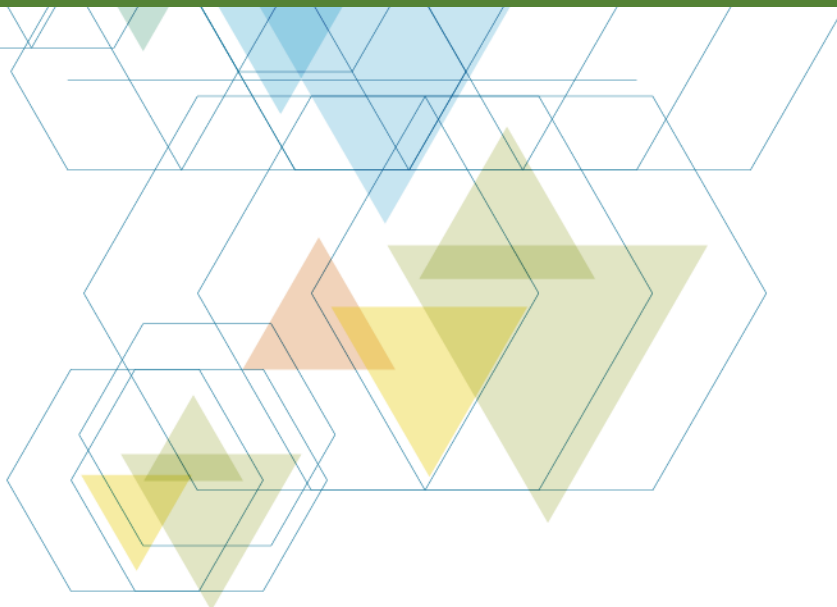
Acknowledgments: Marta Silva wishes to thank FCT for the Ph.D. Grant (10.54499/2020.05266.BD). M.A. Faria thanks FCT the researcher contract CEECINSTLA/00029/2022. This work was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (PREDICT-CARE project, grant agreement No 950050). This research was funded by Fundação para a Ciência e a Tecnologia (FCT) in the framework of the SALIVA+ (2022.08978.PTDC). This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

References:

- [1] M. Bhaswant, D.K. Shanmugam, T. Miyazawa, C. Abe, T. Miyazawa, *Molecules*, 28 (2023).
- [2] D. Del Rio, A. Rodriguez-Mateos, J.P. Spencer, M. Tognolini, G. Borges, A. Crozier, *Antioxid. Redox Signal.*, 18 (2013) 1818–1892.
- [3] L. Bresciani, G. Di Pede, C. Favari, L. Calani, V. Francinelli, A. Riva, et al., *Food Res. Int.*, 141 (2021) 110137.
- [4] M. Dall'Asta, L. Calani, M. Tedeschi, L. Jechiu, F. Brighenti, D. Del Rio, *Nutrition*, 28 (2012) 197–203.
- [5] G. Di Pede, L. Bresciani, F. Brighenti, M.N. Clifford, A. Crozier, D. Del Rio, et al., *Mol. Nutr. Food Res.*, 66 (2022) 2101090.



Sustainability and Circular Economy



Roll-Pulp: Closing the loop on apple juice coproducts

Bárbara Maurício¹, Raquel Nunes da Silva^{2,3*}, Jorge Vieira^{4,5}, Irina Ribeiro Alves⁶, Maria João Alegria⁶, Rui Rodrigues^{4,5}, Ana Fernandes³, Iva Fernandes^{2,3}, António Vicente^{4,5}, Nuno Mateus^{2,3}, Victor de Freitas^{2,3}

¹ Faculty of Engineering, University of Porto, s/n, R. Dr. Roberto Frias, 4200-465 Porto, Portugal

² Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Rua do Campo Alegre 689, 4169-007 Porto, Portugal

³ LAQV-REQUIMTE, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Rua do Campo Alegre 689, 4169-007 Porto, Portugal

⁴ CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

⁵ LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

⁶ SUMOL+COMPAL Marcas S.A, Estr. Portela 24, 2790-179 Carnaxide, Portugal

* raq.silva@fc.up.pt

This study aimed to valorise apple juice pulp by developing an apple roll-pulp snack. Cherry and bilberry extracts were tested for colour improvement, while ingredients like agave syrup and maltodextrin were added to modulate the product flavour and texture. Polyphenols content and antioxidant stability were assessed.

The roll-pulp retained 80% and 66% of its polyphenol content (Folin-Ciocalteu and HPLC quantification, respectively), with antioxidant activity retention of 84% (DPPH) and 90% (FRAP). This stability confirms that the dehydration process minimally degrades bioactive compounds,¹ preserving their health benefits.²

Among the different drying methods studied. Dehydration yielded the best texture, while agave syrup enhanced flavour and appearance. Agave syrup and maltodextrin improved texture by reducing hardness, chewiness, and gumminess, compared to just roll-pulp of 100% pulp, while a water activity of 0.60 reflecting a potential microbiological stability. These findings highlight the role of ingredients, processing, and additive interactions in optimizing the nutritional, physicochemical, and sensory qualities of apple pulp snacks. A survey to assess the agreeability and pleasantness of the product was also conducted with potential consumers.

This study explores upcycling apple juice pulp into a nutritious snack that retains bioactive compounds and antioxidant activity.

Acknowledgments: *This work received support and help from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020), through the project UID/50006 and through national funds and project Agenda VIIAFOOD (n.o C644929456-00000040), funded by the PRR - Recovery and Resilience Plan and by the European NextGeneration EU Funds. This work received financial support from FCT/MCTES (UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020) through national funds.*

References:

- [1] Vega-Gálvez, K. Ah-Hen, M. Chacana, J. Vergara, J. Martínez-Monzó, P. García-Segovia, R. Lemus-Mondaca, K. Di-Scala, Food Chem, 132 (2012) 51.
- [2] D. D. Rio, A. Rodriguez-Mateos, J. P. E. Spencer M. Tognolini, G. Borges, and A. Crozier, Antioxid Redox Sign, 18 (2013) 1818

Food by-products recycling towards production of eco-friendly pH-responsive membranes using natural-inspired dyes

Mariana Cunha¹, Victor de Freitas¹, Idalina Gonçalves², João M. M. Rodrigues², Luís Cruz^{1*}

¹ REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

² CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro – Campus

Universitário de Santiago, 3810-193 Aveiro, Portugal

* luís.cruz@fc.up.pt

The recovery of food by-products for developing innovative materials offers a sustainable approach to addressing global challenges, namely regarding zero waste and environmental conservation. Among these innovations, pH-responsive membranes or films fabricated from food byproducts, such as starch, chitosan, alginate, and other biodegradable polymers, have emerged as promising tools for monitoring food shelf life. These membranes integrate natural, pH-sensitive pigments capable of detecting spoilage through colorimetric changes triggered by shifts in pH levels, which result from the release of volatile compounds during food degradation. In this work, we were able to covalently link two different pH-responsive natural-inspired dyes (10-azidopropyl-4'-hydroxypyranoflavylum and 4'-hydroxy-10-aminoethylcabamoylpyranoflavylum) into alkyne-modified chitosan and aldehydemodified potato wash starch, respectively, by different techniques such as Layer-by-Layer assembly technology and casting. Both free dyes and pH-responsive membranes were fully characterized and evaluated using buffer solutions at different pH levels and biogenic amine-enriched model solutions, which demonstrates the feasibility and effectiveness of these membranes as sensitive materials for food spoilage monitoring.

Acknowledgements: Mariana Cunha acknowledges FCT for the PhD grant (2024.01342.BD). Luís Cruz (2023.07923.CEECIND) and João M. M. Rodrigues (2023.07239.CEECIND) gratefully acknowledge FCT for their research contracts.

Funding: This work received financial support from the PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UID/50006.

References:

- [1] Cunha, M., et al., Acidochromic Free-Standing Multilayered Chitosan-Pyranoflavylum/Alginate Membranes toward Food Smart Packaging Applications. *ACS Applied Polymer Materials*, 2024. 6(11): p. 6820-6830.
- [2] Pires, A.S., et al., Colorimetric pH-Responsive Biomaterials Based on Pyranoflavylum-Biopolymer Hybrid Conjugates. *ACS Applied Polymer Materials*, 2022. 4(7): p. 4961-4971.
- [3] Cruz, L., et al., Natural and Synthetic Flavylum-Based Dyes: The Chemistry behind the Color, in *Chemical Reviews*. 2022, American Chemical Society. p. 1416-1481

Development of carbon dot-embedded polymeric films for intelligent packaging applications

Silva, Pedro Miguel^{1,*}, Azevedo, Ana Gabriela¹, Souza, Victor¹, Brotas, Graça², Filipe, Tiago², Pastrana, Lorenzo¹, Cerqueira, Miguel Ângelo¹

¹ International Iberian Nanotechnology Laboratory, Av. Mte. José Veiga s/n, 4715-330, Braga, Portugal

² Silvex - Indústria de Plásticos e Papéis, S.A., Rua das Camélias nº 7, 2130-233, Benavente, Portugal

* pedro.m.silva@inl.int

Active and intelligent packaging technologies offer promising solutions to address current and important issues in the food system, among which are food waste reduction (1), foodborne diseases (2) or, food fraud (3). The use of these technologies can lead to the improvement of the traceability, detection of contaminants in food, investigation of contamination outbreaks, and the combat of counterfeited products, which have a crucial role in ensuring the safety and quality of packaged food. Carbon dots (CDs) emerge as a novel and powerful solution in the development of intelligent and active packaging, due to their exceptional optical properties, high biocompatibility, and versatility (4). Their use can enable enhanced monitoring and safety in food supply chains via their integration into packaging systems.

In this work, bio-based CDs were prepared using the hydrothermal synthesis method, sodium alginate (2 %, 32.5 mL) and urea (2.5 M, 10 mL), at 180 °C for 11 h. The produced CDs presented a quantum yield of 5.1 %, an excitation wavelength of around 330 nm, and an emission wavelength of around 410 nm. Three solutions of increasing CDs concentration were produced and were incorporated into porous polyethylene (PE) by mixing in a horizontal mixer, and extruded via blown film extrusion, with virgin PE, to yield thin films (~0.035 mm). Produced PE films showed no significant differences regarding color ($\Delta E < 1$) and mechanical analysis. Unlike the PE control film, the PE CD loaded films displayed fluorescence under UV light (~365 nm, Figure 1). The developed CD-loaded films can produce fluorescence under UV light, while remaining transparent under visible light, demonstrating potential for their use in the development of invisible tags for intelligent packaging solutions.

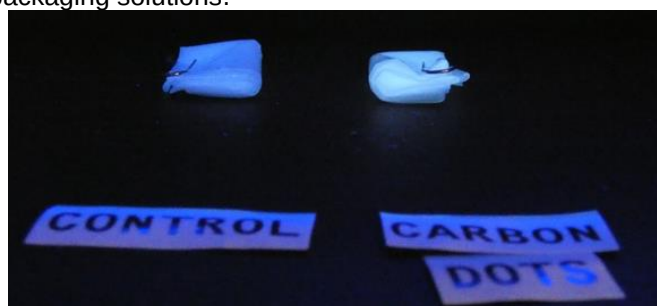


Figure 1. PE film under UV light (~365 nm) (left – control PE film; right – CD-loaded PE film)

Acknowledgments: The authors acknowledge the financial support of the project “Agenda VIIAFOOD: Platform for the Agri-Food Valorisation, industrialization and commercial innovation”, with the reference n.º CC644929456-00000040, co-funded by Component C5 – Capitalisation and Business Innovation under the Portuguese Resilience and Recovery Plan, through the NextGenerationEU Fund.

References:

- [1] United Nations, Food Loss and Waste Reduction | United Nations (2024), (available at <https://www.un.org/en/observances/end-food-waste-day>).
- [2] 2.Sarno et al., J Food Prot. 84, 2059–2070 (2021).
- [3] Giannakas et al., Annu Rev Resour Economics. 15, 85–104 (2023).
- [4] Chung et al., Polymers (Basel). 13, 1598 (2021).

Harnessing black soldier fly larvae for sustainable food waste valorisation and pathogen bioremediation

Joana Oliveira¹, Carolina Ligeiro¹, Rafaela Fantatto^{1,3}, Clarice Souza^{1,3}, Miguel L. Grilo^{1,2}, Carina Carvalho¹, Alexandre Trindade¹, Daniel Murta^{1,3}, Ricardo Assunção^{1,4}

¹Egas Moniz Center for Interdisciplinary Research (CiiEM); Egas Moniz School of Health & Science, 2829-511 Caparica, Almada, Portugal

²MARE–Marine and Environmental Sciences Centre/ARNET–Aquatic Research Network, Ispa–Instituto Universitário de Ciências Psicológicas, Sociais e da Vida, Rua Jardim do Tabaco 34, Lisbon 1149-041, Portugal

³Ingredient Odyssey SA – EntoGreen, Santarém, Portugal

⁴National Institute of Health Dr. Ricardo Jorge, Food and Nutrition Department, Av. Padre Cruz, 1649-016 Lisboa, Portugal
joliveira@egasmoniz.edu.pt

Food waste is a pressing challenge for public health and environmental sustainability, with an estimated 59 million tonnes generated annually in the European Union [1]. This substantial waste compromises the resilience of agrifood systems, intensifies food insecurity, and hampers efforts toward achieving a circular economy [2]. The insect Black soldier fly larvae (BSFL) present a promising and innovative approach to addressing these issues by efficiently converting organic waste into valuable outputs, such as high-protein animal feed and fertilisers [3]. Despite the industrial valorisation of agricultural by-products, BSFL's potential to fully utilise food waste remains subject to some legal restrictions [4].

This study, conducted in collaboration with Ingredient Odyssey SA – EntoGreen, investigated the suitability of food waste as a substrate for BSFL production and assessed the larvae's bioremediation potential for reducing foodborne pathogens. An industrial-scale trial was performed over 14 days, using 114 test units with food waste-based substrates and 144 control units with Gainesville Diet, a standard insect feed. Two tonnes of food waste sourced from restaurants in Santarém, Portugal, were collected and prepared by removing physical contaminants such as plastic, cutlery, and cans. The food waste was then mixed with wheat bran, and its moisture was adjusted to 70% to create an optimal substrate. Measurements, including larval weight and substrate temperature, were taken every other day, while microbiological analyses followed ISO standards to detect pathogens and enumerate microorganisms.

Results showed that food waste substrate significantly enhanced larval growth and bioconversion rates while bioremediating pathogens, including *Bacillus cereus*, *Vibrio spp.*, and *Salmonella spp.* These results highlight the potential of BSFL to transform food waste into valuable resources while mitigating microbial risks, demonstrating a scalable and sustainable solution for food waste valorisation and contributing to a circular economy.

Acknowledgments: This study is funded by a PhD grant by Fundação para a Ciência e Tecnologia (grant number 2022.13540.BDANA); by the Insectera Agenda (<https://www.insectera.pt>) and by CiiEM Research Grant “Flywaste”, through Project UIDB/04585/2020, funded by FCT.

References:

- [1] Eurostat, https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Food_waste_and_food_waste_prevention_-_estimates (2024).
- [2] United Nations, <https://www.un.org/en/observances/end-food-waste-day> (2023).
- [3] Singh, A., Kumari, K., Journal of Environmental Management, 251 (2019) 109569.
- [4] EFSA, EFSA Journal, 13 (2015) 10.

Olive Pits: From agriculture waste to a value product

Catarina Basto-Silva^{1*}, Bruno Silva¹, Paula Allen², Sofia Lourenço², Miguel Figueiredo³, Lúcia Figueiredo⁴, Patrícia Gomes⁵, Catarina Faria¹

¹ PIEP – Centre for Innovation in Polymer Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal

² Sovena Portugal Consumer Goods, S.A., Rua Dr. António Loureiro Borges nº 2 Edifício Arquiparque 2, 3º andar, 1495-131 Algés, Portugal

³ Safiplás - Injeção de Plásticos Lda, Rua da Indústria - Pavilhão 21 e 22 Vilarinho das Cambas Apartado 363, 4760-758 Vila Nova de Famalicão, Portugal

⁴ Flexaco – Concentrados e Aditivos Plásticos, S.A., Zona Industrial de Albergaria-a-Velha, 3850-184 Albergaria-a-Velha, Portugal

⁵ Tratolixo - Tratamento de Resíduos Sólidos, E.I.M., S.A., Estrada 5 de Junho, Nº1 – Trajouce, 2785-155 São Domingos de Rana, Portugal

* *catarina.silva@piep.pt*

On the Mediterranean coast, the olive industry generates millions of tonnes of waste annually, including olive pits. These residues, known for their hardness, are ideal as reinforcements in composite polymeric materials, transforming waste into valuable resources [1]. The Olive Pit project leverages this potential by developing biodegradable shoe shapers using olive pits and polylactic acid (PLA), offering an alternative to traditional fossil-derived shoe forms. By achieving bio-compostability, the project aims to close the product's life cycle, repurposing it as fertiliser to support olive production.

This study compares the environmental impacts of conventional shoe shapers made from acrylonitrile butadiene styrene (ABS) or polypropylene (PP) with novel biodegradable shoe shapers developed in the project. Various formulations were produced, incorporating differing proportions of olive pits, PLA, and additives, while maintaining the rheological properties essential for the product's success.

A prospective Life Cycle Assessment (LCA) was performed in compliance with ISO14040:44 standards [2, 3], adopting a cradle-to-gate approach. The declared unit was defined as the raw materials needed for one pair of shoe shapers. Environmental impacts were assessed using the ReCiPe Midpoint Hierarchist perspective method (v 1.09) available on SimaPro software (v 9.6.0.1). Primary data were provided by PIEP and Safiplás, and secondary data were sourced from the Ecoinvent database (v 3.10).

Initially, virgin PLA formulations were tested to create a sustainable shoe shaper, but high environmental impacts led to a shift towards recycled PLA. Results show that formulations incorporating olive pits achieve lower environmental impacts than baseline materials, demonstrating the advantages of recycled and bio-based materials. Future research should focus on the biodegradability benefits at the end-of-life stage, which could promote the growth of new olive trees and foster a sustainable, regenerative approach within the olive industry.

Acknowledgments: This research was supported by the NextGenerationEU programme under the Recovery and Resilience Facility Plan for Portugal (Code RE-CO5-i01.02 Sustainable Plastics, WP4, PPS14).

References:

- [1] Burgos Pintos, P., Maturi, M., Sanz de León, A., & Molina, S. I. (2024). Development of polymer composites using surface-modified olive pit powder for fused granular fabrication. *Polymers*, 16(21), 2981. <https://doi.org/10.3390/polym16212981>.
- [2] International Organization for Standardization. (2006). ISO 14040:2006 Environmental management—Life cycle assessment—Principles and framework.
- [3] International Organization for Standardization. (2006). ISO 14044:2006 Environmental management—Life cycle assessment—Requirements and guidelines.

[S&EC 06]

Functional Muffins with Lime Extract: A Step Toward Sustainable Food Innovation

Ana Rita Soares Mateus^{1,2,3,4}, Carina Almeida^{1,5,6}, Angelina Pena^{2,3}, Ana Sanches Silva^{2,4,7*}

¹ National Institute for Agricultural and Veterinary Research (INIAV), I.P., Rua dos Lagidos, Lugar da Madalena, 4485-655 Vairão, Vila do Conde, Portugal (ARSM: anarita.mateus@iniav.pt)

² Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de St^a Comba, 3000-548 Coimbra, Portugal. (AP: apena@ci.uc.pt, ASS: asanchessilva@ff.uc.pt)

³ LAQV, REQUIMTE, Laboratory of Bromatology and Pharmacognosy, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de St^a Comba, 3000-548 Coimbra, Portugal.

⁴ Center for Study in Animal Science (CECA), ICETA, University of Porto, Apartado, 55142 Porto, Portugal

⁵ LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal 10

⁶ AliCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

⁷ Associate Laboratory for Animal and Veterinary Sciences (Al4AnimalS), 1300-477 Lisbon, Portugal asanchessilva@ff.uc.pt

Lime (*Citrus aurantifolia*) by-products, generated in large quantities by the citrus processing industry, are rich in bioactive compounds such as phenolics and flavonoids [1]. These compounds possess antioxidant and health-promoting properties [2], making lime by-products a promising ingredient for developing functional foods while addressing food waste and sustainability challenges [3]. This study aimed to evaluate the incorporation of lime by-product extract at different concentrations (1%, 1.5%, and 2%) into muffin formulations (corresponding to control, M1, M2 and M3 formulations) to assess its effects on functional and sensory properties.

Muffins were analyzed for their antioxidant properties, individual phenolic compounds (using Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry - UHPLC-ToF-MS), lipid oxidation stability (via Thiobarbituric Acid Reactive Substances Assay - TBARS), and sensory acceptance. The sensory evaluation included 9-point hedonic scale and Just About Right scales to assess consumer preferences.

The findings demonstrated that baking partially degraded phenolic compounds, yet significant levels of bioactive compounds, particularly flavonols and flavanones, were retained. For example, the level of hesperidin ranged from 0.136 to 14.41 µg/g (in M3) in doughs and ranged from 0.006 to 13.6 µg/g in muffins, with M3 showed the highest total phenolic content (96.51 µg/g). Lipid oxidation stability varied among formulations, with M1 showing the highest stability and consumer acceptance. In contrast, M3 exhibited a pro-oxidant effect and was associated with increased bitterness, resulting in lower sensory scores. These results highlight the importance of optimizing extract concentrations to balance functional benefits and sensory appeal in baked goods. Incorporating lime by-product extract enhances muffins' functional potential and aligns with sustainability goals by valorizing food waste. Future research could explore the rheological and physical characteristics of muffins, as well as strategies to maximize phenolic compound retention during baking. This research contributes to functional food innovation and waste valorization, providing actionable insights for the development of sustainable, health-promoting baked products.

Acknowledgments: This work was financially supported by the research project ValICET (PRIMA/0001/2020) - Valorise foods and Improve Competitiveness through Emerging Technologies applied to food by products within the circular economy framework (section 2 PRIMA project) funded in Portugal by Foundation for Science and Technology (FCT) (DOI 10.54499/PRIMA/0001/2020). The work was supported by UIDB/00211/2020 with funding from FCT/MCTES through national funds. A.R.S.M. would like to thank to FCT for her fellowship (2023.04705.BDANA).

References:

- [1] R. S. Mateus, S. Mariño-Cortegoso, S. C. Barros, R. Sendón, A. Pena & A. Sanches-Silva, *Innovative Food Science & Emerging Technologies*, 95 (2024) 103737
- [2] T. D'Amore, M. Chaari, G. Falco, G. De Gregorio, N. Zarái Jaouadi, D. Ali, T. Sarkar & S. Smaoui, *Biocatalysis and Agricultural Biotechnology*, 58 (2024) 103163.
- [3] N.J. Temple, *Frontiers in Nutrition*, 9 (2022) 957516.

Aligning present and future agrifood systems: Integrating sustainability and health for better choices

Ricardo Assunção^{1,2*}, Alexandra Bento¹

¹ National Institute of Health Dr. Ricardo Jorge, Food and Nutrition Department, Lisbon, Portugal

² Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science, Almada, Portugal

* *ricardo.assuncao@insa.min-saude.pt*

The transition to sustainable food systems is essential to address global challenges such as climate change, biodiversity loss and public health crises [1]. Current food production and consumption patterns contribute to environmental degradation, while dietary choices strongly influence health outcomes and planetary sustainability [2]. Supporting dietary decisions with robust scientific evidence is therefore essential for meaningful and informed change [3].

This work aims to discuss the intersection of food system transitions, dietary health impacts and sustainability, highlighting the importance of a comprehensive evidence-based approach to guide decisions at individual and systemic levels. By integrating methodologies such as Risk-Benefit Assessment (RBA) and Life Cycle Sustainability Assessment (LCSA), this study provides an approach to evaluating foods, dietary patterns and food choices. RBA considers risks (e.g. food contaminants) and benefits (e.g. beneficial nutrients), while LCSA assesses the environmental, social and economic dimensions of food production and consumption.

Inspired by the ALTERNATIVA project [3], the integration of these two perspectives, complemented by additional tools such as Multi-Criteria Decision Analysis (MCDA), will be explored and synthesised. The combination of these methodologies enables a dual-angle approach that quantifies health outcomes alongside sustainability metrics, providing a framework for balancing dietary health benefits with sustainable food systems.

This approach not only informs recommendations for healthier and more sustainable diets but also supports policymakers and stakeholders in developing strategies for food system transformation. By bridging gaps between food system actors and aligning public health goals with environmental sustainability, this evidence-based framework promotes a holistic vision for the agri-food sector and empowers consumers and stakeholders to make better choices that benefit both humans and the planet.

References:

- [1] W. Willett et al., The Lancet, vol. 393, no. 10170, pp. 447–492, Feb. 2019.
- [2] J. Fanzo and L. Miachon, Anthropocene, vol. 42, p. 100381, Jun. 2023.
- [3] R. Assunção et al., EFSA Supporting Publications, vol. 21, no. 10, Oct. 2024.

From grape pomace to valuable products: Sequential fractionation using ohmic heating and subcritical hydrolysis

Bianca Marques^{1,*}, Natacha Fontes², José A. Teixeira^{1,3}, Cristina M.R. Rocha^{1,3}

¹Centre of Biological Engineering, University of Minho, Braga, Portugal

² Sogrape Vinhos S.A., Avintes, Portugal

³ LABBELS - Associate Laboratory, University of Minho, Braga, Portugal

* *bianca_marques_93@hotmail.com*

Grape pomace (GP) is the main viticultural by-product, representing up to 25 % of the overall grapes used during the winemaking process [1]. GP composition is mainly cellulose, hemicellulose, lignin, pectin and polyphenols [1,2,3], that could be valorised into useful products. This work aimed to exploit a "zero waste biorefinery cascade", using green technologies, including ohmic heating (OH) and subcritical hydrolysis (SWH). Both technologies have been used for GP's valorization [2,3]. However, in this work GP was fractionated in a sequential methodology of OH followed by SWH, that, to the best of the authors' knowledge, there is no current reported data about the use of this combined strategy. All fractions produced will be further applied in development of new products or to improve existing ones.

Red and white GP were dried at 45 °C and milled. OH was performed using a ratio of 1:20 (g_{dw} GP: mL ethanol at 45 %). Extraction occurred at 80 °C, using an electrical field of 3.5 V/cm, during 60 min. Each SWH run used two of OH solid fractions and 400 mL of dH₂O, and occurred at 180 °C. At the end of each treatment, the extracts were filtered and the solid fractions were dried at 60 °C, while the liquid extracts were preserved at -20 °C for further analysis. The resulting fractions were analysed for their sugars and polyphenols content.

Results showed that OH resulting liquid extracts were mainly polyphenol-enriched and SWH liquid extracts had high amounts of sugars and polyphenols. The resulting solid fractions were mainly composed by cellulose and lignin. Moreover, all liquid extracts displayed antioxidant activities.

The sequential OH-SWH fractionation of GP shown as promising strategy for its integral valorisation, and the resulting extracts could have potential applications in human nutrition, cosmetics and biopolymers production.

Acknowledgments: We would like to thank to Sogrape, Vinhos S.A. for providing the wine by-products. This work was financially supported by Fundação para a Ciência e Tecnologia (FCT) under the scope of the strategic funding of UID/BIO/04469/2019 unit and LA/P/0029/2020. Bianca Marques is recipient of a doctoral fellowship supported by FCT (2023.04457.BDANA).

References:

- [1] T. Atatoprak, M.M. Amorim, T. Ribeiro, M. Pintado, A.R. Madureira, Food Chemistry: Molecular Sciences, 4 (2022)
- [2] M.C. Coelho, S. Ghalamara, R. Pereira, A.S. Rodrigues, J.A. Teixeira, M.E. Pintado. Processes, 11 (2023) 495.
- [3] L.N. Castro, T.L.C.T. Barroso, W.G. Sganzerla, J.M.Costa, F.T. Saia, L.M.S. Colpini, T. Forster-Carneiro, Fuel, 363 (2024)

Sustainable value chains in mountain rural areas: Strategies for a model region

**Catarina Afonso^{1,*}, Violeta Lopes¹, Ana Maria Barata¹, Jorge Miranda²,
Nuno V. Brito^{3,4}, Daniel Raposo⁵, Teresa Paiva^{6,7}**

¹ Banco Português de Germoplasma Vegetal, Instituto Nacional de Investigação Agrária e Veterinária I.P., Braga, Portugal

² ACIBTM/In.Cubo – Associação Centro de incubação de Base Tecnológica do Minho, Arcos de Valdevez, Portugal

³ CISAS, Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Viana do Castelo, Portugal

⁴ 1H-TOXRUN - One Health Toxicology Research Unit, University Institute of Health Sciences, CESPU CRL., Gandra, Portugal

⁵ ReThink - Centre for Research in Design for the Territory, Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal

⁶ BRIDGES - Biotechnology Research, Innovation and Design for Health Products, Polytechnic of Guarda, Guarda, Portugal

⁷ NECE - Research Centre in Business Sciences, University of Beira Interior, Covilhã, Portugal

* catarina.afonso@iniav.pt

Mountain Rural Areas (MRAs) have always faced challenges due to their biogeographical location. Nowadays, MRAs vitality is not only weakened by their particular vulnerability to climate change¹, but also by significant demographic aging and fragility of agri-food systems, which are often based on low-productive and low-value-added practices and an unskilled/underqualified workforce - typical of low-density territories². Although positive discrimination measures for these territories have been implemented in recent years, their impact remains limited in attracting population and investment and, thus, reversing the demographic circumstances of these areas³. Effective strategies for attracting people to MRAs should emphasize MRAs' unique territorial capital and identities, valorise their regional endogenous resources, and leverage their historical, cultural, and natural heritages, creating distinctive solutions that improve current residents' lives while attracting new inhabitants. These strategies require local participation for addressing place-specific challenges of MRAs⁴. Simultaneously, it is imperative to enhance the sustainability and the competitiveness of agricultural and agri-food sectors of MRAs through technological and social innovation⁵.

The Interior+ project aims at the sustainable revitalization of MRAs of Alto Minho, Beira Baixa, and Beiras e Serra da Estrela. For that, Interior+ is building a Model Region for the valorisation of regional endogenous resources of MRAs, to be replicated in other MRAs. Here, we present the Interior+ strategy for a Model Region. This strategy firstly implements comprehensive surveys to agri-foods producers to gather first-hand information about their needs, aspirations, and capacities for innovation. Additionally, it includes a full assessment of key socio-economical, technological and environmental challenges and opportunities of agri-foods value chains identified in each target MRA. Finally, it mobilizes resources, transfers knowledge and encourages the implementation of technological and social solutions in order to create resilient and prosperous MRAs, aligned with the EU Rural Agenda priorities.

References:

- [1] C. Adler, P. Wester, I. Bhatt, C. Huggel, G.E. Insarov, M.D. Morecroft, V. Muccione, A. Prakash, in *Climate Change 2022: Impacts, Adaptation and Vulnerability*, H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (Eds.), Cambridge, UK and New York, NY, USA, Cambridge University Press, 2022, 2273–2318.
- [2] Globalização, Agricultura e Zonas Rurais Desfavorecidas, M. B. Moreira, Oeiras, Celta Editora, 2001.
- [3] M. A. Almeida, *Revista Galega de Economía*, 29(2) (2020) 1-17.
- [4] T. Dax, *Neoendogenous Rural Development in Mountain Areas*, in *Neoendogenous Development in European Rural Areas*, E. Cejudo, F. Navarro (Eds.), Springer Geography, Springer, 2020.
- [5] J. McElDowney, in *Long-term vision for rural areas: European Commission communication*, European Parliamentary Research Service (Eds.), Brussels, European Union, 2021.

Sustainable production of lignin nanoparticles from grape stalks via sonication

Ana C. Cassoni^{1,*}, Marta Vasconcelos¹, Manuela Pintado¹

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina –
Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

* *acassoni@ucp.pt*

Lignin nanoparticles represent an emerging class of sustainable nanomaterials that can improve the valorisation of this abundant biopolymer through new applications [1]. This study focused on optimizing the production of lignin nanoparticles through sonication, using as few chemicals as possible while achieving small particle sizes and maintaining colloidal stability. To avoid aggregation and sustain stability, two surfactants were tested. Nanoparticles were characterized using Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) to evaluate their size distribution and morphology. Through the optimization of sonication parameters (lignin load, amplitude, and time), spherical lignin nanoparticles with sizes ranging from 200 to 300 nm were obtained. TEM confirmed the particle size measurements obtained through DLS and provided information about particle morphology and aggregation. Results show that adding surfactant is essential to avoid aggregation of nanoparticles and maintain their stability. The developed methodology demonstrates a simple and efficient approach for producing spherical lignin nanoparticles, contributing to the advancement of lignin valorisation strategies and expanding potential applications of this biopolymer.

Acknowledgments: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UIDB/50016/2020. Funding for author Ana C. Cassoni was provided by Fundação para a Ciência e a Tecnologia – Portugal (via the PhD fellowship ref. SFRH/BD/143198/2019).

References:

- [1] S. Beisl, A. Friedl. & A. Miltner, International Journal of Molecular Science, 18 (2017) 2367.



Comparative assessment of solid and dissolved lignin from grape stalks for antibacterial applications

Ana C. Cassoni^{1,*}, Sara A. Cunha¹, Marta Vasconcelos¹, Manuela Pintado¹

¹ CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

* acassoni@ucp.pt

The valorisation of agricultural wastes by extracting bioactive compounds represents a promising approach in sustainable biotechnology. This study investigated the antibacterial properties of lignin extracted from grape stalks, evaluating its efficacy in both solid and dissolved forms against three relevant bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Lignin was isolated from grape stalks using deep eutectic solvents (DES) [1]. The extracted lignin was subjected to micronization and dissolved using Tween 80. The assays were conducted by mixing the bacterial inoculum with lignin extracts in 24-well plates maintained under constant agitation. After 24 hours, serial dilutions were performed in 0.1% peptone water and planted on Mueller-Hinton agar to determine CFU/ml. Results show that solid lignin has antimicrobial activity against *S. aureus* but is ineffective for the other two bacterial strains. Dissolved lignin had better performance and had an antimicrobial effect against all three bacteria, especially *P. aeruginosa* and *S. aureus*. These results show that lignin bioavailability is improved when dissolved, as expected. Nonetheless, it is interesting to notice that solid lignin also has some potential, namely against *S. aureus*. This fact is essential because lignin is difficult to dissolve and often requires harsh chemicals that can limit its applicability. For example, using solid lignin with antimicrobial properties could be important for biological applications.

In conclusion, this study shows the potential of lignin extracted from grape stalks as an antibacterial agent, demonstrating enhanced efficacy in its dissolved form, while revealing a promising activity of solid lignin against *S. aureus*, paving the way for sustainable and versatile applications in biotechnology.

Acknowledgments: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project UIDB/50016/2020. Funding for author Ana C. Cassoni was provided by Fundação para a Ciência e a Tecnologia – Portugal (via the PhD fellowship ref. SFRH/BD/143198/2019).

References:

- [1] A.C. Cassoni, I. Mota, P. Costa, M. Vasconcelos, & M. Pintado, International Journal of Biological Macromolecules, 220 (2022) 406-414.

Linking Landscape, Environment, Agriculture and Food towards a more sustainable agri-food value chain

Joana Costa^{1,*}, David Fangueiro¹, Luis Goulão¹, Anabela Raymundo¹, Isabel Sousa¹

¹LEAF-Linking Landscape, Environment, Agriculture and Food Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Lisboa, Portugal

* joanacosta@isa.ulisboa.pt

LEAF (Linking Landscape, Environment, Agriculture and Food) is a research centre hosted by Instituto Superior de Agronomia - ULisboa, with the mission to conduct comprehensive research to advance transformative knowledge by linking landscape, environment, agriculture and food, followed by knowledge transfer through education, training and science dissemination, contributing to the sustainable progress of society.

LEAF creates knowledge from landscape, soil and water management, through plant, animal and food sciences, to food and feed processing, co-producing innovation with stakeholders from the agricultural sector, natural resources management, and agro-food industry, to respond to societal needs, with a strong contribution to Planetary Health, and covering the whole food value chain. LEAF is therefore uniquely positioned to generate knowledge through a holistic approach. Our 3D dimensions are defined by climate neutrality, bioeconomy and Planetary Health, including smart and digital drivers. This demonstrates our global value chain approach from the territory to people, using SMART thinking at different scales, from biomolecules and cells to precision agriculture and landscape planning.

LEAF is strongly committed to support the ongoing disruptive societal transitions towards sustainable development, driven by AI integration, adoption of clean energy, and ensuring sustainable and affordable food systems for healthy diets. It is attentive to societal needs on responsible production and consumption, driven by the current demographic transitions, including human mobility, growing peri-urban populations and ageing.

Our strong background in Agricultural and Environmental Sciences, together with our commitment to contribute to societal needs resulted in the identification of Focal Strategic Areas of research for 2025-29, to be presented herein. These are a) Landscape Planning, Design & Policies, b) Soil & Water Management, c) Inputs' Reduction for "Greener Ways of Farming", d) Grapevine & Wine Value Chain, and e) Transitions to Sustainable, Healthy and Affordable Food & Feed.

Acknowledgments: This work was funded by national funds through FCT – Fundação para a Ciência e a Tecnologia, I.P., under the projects UIDB/04129/2020 and UIDP/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food Research Center.



Valorizing saffron by-products as a source of an anthocyanin-rich natural colourant

Leonor Teixeira da Costa^{1,2,*}, Ana N. Nunes^{1,2}, Maria Rosário Bronze^{1,2,3}, Cátia Saldanha do Carmo^{1,2} Ana Teresa Serra^{1,2}

¹IBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901, Oeiras, Portugal

²Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

³IMED, Faculdade de Farmácia da Universidade de Lisboa, Av. das Forças Armadas, 1649-019 Lisboa, Portugal

*leonor.costa@ibet.pt

The global shift towards health-conscious and environmentally sustainable practices has intensified the demand for natural pigments in the food industry. Natural colourants are recognized as promising clean-label solutions, aligning with consumer preferences [1,2]. Saffron by-products (SBP), which represent approximately 93% of the biomass generated during saffron production, offer significant potential. Being predominantly composed of tepals, SBP are rich in anthocyanins and flavonols with a notable antioxidant capacity [3,4], representing a valuable source for producing natural functional colourants. This study aimed to valorise SBP, focusing on the optimization of a hydroalcoholic extraction process to maximize the anthocyanin yield (AY), extraction yield (EY), and total anthocyanin content (TAC).

Response surface methodology, following a Central Composite Face-Centered was used, varying the parameters: solid-to-solvent ratio (1:125 – 1:40g/mL), ethanol content (0-100%, pH2), and extraction time (10-60 min). Seventeen extracts were produced and characterized in terms of TAC (pH differential method and HPLC-DAD-UV), antioxidant activity (ORAC), and colour properties (CIELAB method). The optimal extraction conditions were identified, enabling an EY of 71.1%, an AY of ≈100%, and a TAC of 1.13g/100g_{d.ext.}. The ethanol content was the parameter with the most significant impact in all responses. Increasing ethanol content led to a decrease in EY, AY, TAC, and ORAC. The antioxidant activity of the extract obtained at optimal conditions was 2234 μmol TEAC/g of dried extract with kaempferol-3-sophoroside, delphinidin-3,5-diglucoside, and kaempferol-3-sophoroside-7-glucoside as the main contributors. A significant reduction in the extraction time and solid-to-solvent ratio (1:125g/mL) was achieved in this work compared to previous studies [5], while maximizing the recovery of SPB-anthocyanins.

This work supports the development of an alternative and sustainable anthocyanin-rich extract from SBP with improved bioactivity for potential application as a natural colourant in the food industry.

Acknowledgments: The authors acknowledge the PRR through the Agenda VIIAFOOD – Plataforma para a Valorização, Industrialização e Inovação comercial para o Agroalimentar”, framed in the notice nº 02/C05-i01/2022. The authors also acknowledge Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020). The authors also acknowledge FCT for the PhD grant of Leonor Costa (2021.08117.BD) and the CEEC grant of Teresa Serra (CEEIND/04801/2017). The authors also acknowledge Lagar do Clavijo for providing the samples of saffron by-products.

References:

- [1] R. Cortez, D. A. Luna-Vital, D. Margulis and E. Gonzalez de Mejia. Comprehensive Reviews in Food Science and Food Safety, 16 (2017), 180-198
- [2] P. Cazón and A. Sanches Silva. Green and Sustainable Chemistry. 47 (2024), 100929
- [3] J. Serrano-Díaz, A. M. Sánchez, M. Martínez-Tomé, P. Winterhalter & G. L. Alonso, Journal of Food Composition and Analysis, 31 (2013), 101-108
- [4] L. Lotfi, A. Kalbasi-Ashtari, M. Hamedí & F. Ghorbani, J Food Sci Technol., 52 (2015), 813–821
- [5] K. M. Khazaei, S. M. Jafari, M. Ghorbani, A. Hemmati Kakhki & M. Sarfarazi, Food Anal. Methods, 9 (2016), 1993–2001

Circular Animal Protein Hydrolysates: a comparative approach of functional properties

**Monteiro, M.^{1,2*}, Rodrigues-dos-Santos, L.¹⁻⁴, Filipa-Silva, A.¹, Marques, D.A.¹,
Pintado, M.³, Almeida, A.⁴, Valente, L.M.P.^{1,2}**

¹CIIMAR/CIMAR-LA, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Matosinhos, Portugal

²ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal

³Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia

⁴ETSA, Empresa Transformadora de Subprodutos, 2660-119 Loures, Portugal

* marta.monteiro@ciimar.up.pt

The growing interest in novel nutraceutical sources has heightened the demand for efficient and cost-effective biocompound alternatives [1]. In this context, the circular economy presents an abundant supply of low-value proteins that can be enhanced to improve their nutritional and functional properties [2,3]. This study aimed to evaluate five different protein hydrolysates derived from animal by-products processed by ETSA S.A., comparing them to a high-quality fish protein (CPSP90). These hydrolysates were assessed for their proximate composition, amino acid profile, peptide molecular weight distribution, as well as their potential antioxidant activity and role in bacterial growth dynamics.

The hydrolysates exhibited distinct chemical and functional properties reflecting their diverse origins. Crude protein content ranged from 55.1–89.5% DM, with Shark Hydrolysate (SH) and Swine Hydrolysate (SWH) containing the highest protein levels. Essential amino acids were most abundant in the Fish Hydrolysate (FH) while non-essential amino acids were predominant in SH. Insect hydrolysate (IH), showed the highest antioxidant activity among the hydrolysates, which could be connected to the abundance of low molecular weight peptides (<1 kDa) and high content in aromatic amino acids, such as tryptophan and tyrosine. SWH demonstrated the highest solubility across different pH (3-7) and strongly modulating bacterial proliferation, selectively inhibiting the growth of opportunistic pathogens when compared to CPSP90.

In conclusion, the evaluated hydrolysates exhibited distinct chemical and functional properties, reflecting their diverse sources. SWH's superior solubility and selective antibacterial activity, alongside the potent antioxidant capacity of IH underscore their potential as functional ingredients in animal feeds. These findings highlight the value of animal by-products in advancing functional feed solutions within a circular economy framework.

Acknowledgments: This research was supported by the Blue Bioeconomy Pact (C644915664-00000026), for the exercise of activities in the WP6 FEED (Pep4Fish project) through international funds provided by the European Union, and by FCT – Foundation for Science and Technology (FCT) within the scope of UIDB/04423/2020 and UIDP/04423/2020. Luciano Rodrigues-dos-Santos acknowledges FCT for his PhD grant (2023.00947.BDANA).

References:

- [1] Y. Etemadian, V. Ghaemi, A. R. Shaviklo, P. Pourashouri, A. R. Sadeghi Mahoonak, and F. Rafipour, 'Development of animal/ plant-based protein hydrolysate and its application in food, feed and nutraceutical industries: State of the art', *J Clean Prod*, vol. 278, p. 123219, Jan. 2021, doi: 10.1016/J.JCLEPRO.2020.123219.
- [2] T. M. G. Salerno *et al.*, 'Circular Economy in the Food Chain: Retrieval and Characterization of Antimicrobial Peptides from Fish Waste Hydrolysates', *Food Anal Methods*, vol. 17, no. 2, pp. 178–199, Dec. 2023, doi: 10.1007/S12161-023-02543-Z/TABLES/2.
- [3] M. Angulo and M. a C. Márquez, 'Enzymatic Hydrolysis of Meat Waste for a Circular Economy', *Catalysis Research* 2023, Vol. 3, 008, vol. 3, no. 1, pp. 1–26, Feb. 2023, doi: 10.21926/CR.2301008.

Evaluating the Influence of Functional Unit Selection in Life Cycle Assessment: A Bread Case Study

Rui A. dos Reis¹ *, Ana Fonseca², Ana Cláudia Dias², Paula Quinteiro², Vera Fernandes³, Emanuel J. Lourenço¹

¹ INEGI – Institute of Science and Innovation in Mechanical and Industrial Engineering, Rua Dr. Roberto Frias 400, 4200-465 Porto, Portugal

² Centre for Environmental and Marine Studies (CESAM), Department of Environment and Planning, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal

³ Auchan Retail Portugal, Estrada de Paço de Arcos nº48 A, 2770-129, Paço de Arcos, Portugal

* rreis@inegi.up.pt

In recent years, there has been a growing concern on the need for healthier diets and more nutritional food options. Bread, is one of the most widely consumed foods in daily life, has become a focus of research aimed at enhancing its nutritional value [1]. However, the food sector is a major contributor to environmental impacts, including ecosystem degradation, increased greenhouse gas emissions, and land use [2]. Incorporating insect-based products into food production represents a promising strategy for improving the nutritional profiles of various foods, including bread. Currently, the production of insects for the food sector has been increasing significantly in Western countries, creating a new bio-industrial sector and an opportunity to reduce the carbon footprint of consumed proteins in these countries [3].

When developing new food products, it is crucial to evaluate their potential environmental impacts early in the process to identify potential hotspots and challenges before scaling up production [4]. This study investigated the trade-offs when assessing the potential environmental impacts of food items, focusing on how the selected functional unit influences comparative results.

Based on data from the literature, a life cycle assessment study was conducted, considering three functional units: mass of bread, caloric content, and protein content. The results indicate that using a mass-based functional unit yields better environmental outcomes for simpler bread recipes comprising mainly flour, yeast, and water. On the other hand, when nutritional value-based functional units, such as caloric content, are applied, the results shift in favour of bread types that include additional ingredients like vegetable oils and small amounts of animal-based products.

These findings will contribute to developing life cycle approaches to assess the potential environmental, social, and economic benefits or drawbacks of incorporating insect-based products into the food sector.

Acknowledgments: This study is funded by the PRR - Recovery and Resilience Plan and by the NextGenerationEU funds at INEGI, through the scope of the Agenda for Business Innovation “InsectERA” (Project no. 20 with the application C644917393-00000032).

References:

- [1] Sajdakowska, M., Gębski, J., Zakowska-Biemans, S., & Jezewska-Zychowicz, M. (2019). Willingness to eat bread with health benefits: Habits, taste and health in bread choice. *Public Health*, 167, 78–87. <https://doi.org/10.1016/j.puhe.2018.10.018>
- [2] FAO, UNEP, WHO, and WOA. (2022). One Health Joint Plan of Action (2022-2026). Working together for the health of humans, animals, plants and the environment. Rome. <https://doi.org/10.4060/cc2289enX>.
- [3] Acosta-Estrada, A., Reyes, A., Rosell, C., Rodrigo, D., Ibarra-Herrera, C., (2021). Benefits and Challenges in the Incorporation of Insects in Food Products. *Front. Nutr.* 8:687712. <https://doi.org/10.3389/fnut.2021.687712>
- [4] Moura, B., Monteiro, H., Mata, T.M., Iten, M., Martins, A.A., 2022. Environmental life cycle assessment of early-stage development of ergosterol extraction from mushroom bio-residues. *J. Clean. Prod.* 355, 131623. <https://doi.org/10.1016/J>.

Chestnut Burrs: A cellulose source for stabilising pickering emulsions

Joana Lains^{1,2}, Ricardo Santos^{1,2}, Cláudia G. Silva^{1,2}, Madalena M. Dias^{1,2}, M. Filomena Barreiro^{3,4}, Andreia Ribeiro^{1,2,*}, Isabel Martins^{1,2,*}

¹Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials (LSRE-LCM), Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

²ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

⁴Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

* isa@fe.up.pt; asribeiro@fe.up.pt

The rise in the global population has led to increased food consumption, resulting in significant agri-food residues. Portugal, as the 8th chestnut producer in the world [1], generates a significant amount of chestnut by-products, such as shells, burrs and leaves. Trás-os-Montes region, in the northeast of Portugal, comprises 85 % of the national chestnut production [2]. Therefore, the CyChest project seeks to repurpose these residues by extracting valuable compounds and integrating them into high-value-added products. The main objective of this work is to extract cellulose from chestnut burrs and utilise it as a stabiliser for Pickering emulsions (PE).

Cellulose extraction comprises pre-treatments, alkali hydrolysis, and bleaching treatment [3]. The optimisation study of hydrolysis parameters revealed that temperature is the critical factor, processing time has no effect, and a lower alkali concentration is sufficient to remove a significant amount of lignin. The optimisation of the bleaching treatment confirmed that temperature remains the critical parameter, while the choice of bleaching agent influences the cellulose content and crystallinity achieved. The final extract comprises 72% cellulose and 16% lignin, with a whiteness index of 82%. The resulting particles measure 55 µm in size, possess a negative surface charge, exhibit hydrophilic properties, and show a non-organised form of the cellulose fibres with a rough surface and a spherical shape.

A stable PE (20:80 O/W; sweet almond oil) was achieved with 0.8 wt% of cellulose extracted, exhibiting a droplet size of 12 µm and two mechanisms of stabilisation: a network between the larger cellulose particles and adsorption of the smaller particles onto the oil surface. This PE remains stable for a month. This work represents progress toward sustainability and a circular economy by creating emulsion-based products through the valorisation of chestnut burrs, a by-product of chestnut production.

Acknowledgements: This work is financially supported by the Promove Programme through Fundação “La Caixa”, in collaboration with the BPI and Fundação para a Ciência e a Tecnologia (FCT), under the project CyChest - Gestão integrada do ciclo do castanheiro no Parque Natural de Montesinho. This work was also supported by national funds through FCT/MCTES (PIDDAC): LSRE-LCM, UIDB/50020/2020 (DOI: 10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020); and ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020); CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020) and LA SusTEC, LA/P/0007/2021 (DOI:10.54499/LA/P/0007/2020). Andreia Ribeiro acknowledges FCT for her research contract through the individual CEEC – Stimulus of Scientific Employment (DOI: 10.54499/2022.00798.CEECIND/CP1733/CT0009).

References:

- [1] Top 10 Country Production of Chestnuts, in shell. [cited 2024 27 May]; Available from: https://www.fao.org/faostat/en/#rankings/countries_by_commodity.
- [2] J. Gomes-Laranjo, L.-T. Dinis, L. Martins, E. Portela, T. Pinto, M. Ciordia, I. Feito, J. Majada, F. Peixoto, and S. Pereira, Applied photosynthesis, **3** (2012), 47-80.
- [3] L. Chopra, Materials Today: Proceedings, **92** (2023), 233-239.

Extraction and stabilisation of bioactive compounds from chestnut shell residues

Maria João Cabaço¹, Carina A. E. Costa^{2,3}, Alírio E. Rodrigues^{2,3}, Isabel M. Martins^{2,3*}

¹Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

²LSRE-LCM – Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

³ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

*isa@fe.up.pt

Food waste has been regarded as a natural resource of valuable ingredients, such as phenolic compounds [1]. Valorisation of industrial by-products by extraction of bioactive compounds has attracted attention in the last years, as well as their incorporation in cosmetic, food, and pharmaceutical products. *Castanea sativa* Mill. is an abundant chestnut species in the south of Europe, with historical significance and economic relevance nowadays. Chestnut shells are usually wasted despite being rich in polyphenols [2,3].

This work intended to optimise the Soxhlet extraction of chestnut shells, by selecting the most suitable shell condition and extraction solvent using fresh chestnut shells and ones that underwent domestic oven and brulage temperatures. The aim was also to stabilise the extracted compounds by complex coacervation, using biocompatible polymers: gum Arabic and chitosan. Soxhlet extractions were done with whole and ground shells from different pre-treatments and mixtures with different ethanol-water ratios. Folin-Ciocalteu's method was used to quantify the total phenolic content in the extracts. The highest yield of these compounds was obtained with ground shells due to the increased transfer area by grinding. Water and the mixture with 10% ethanol at were the two most effective extraction solvents. Fresh shells had the highest phenolic content while brulage shells had the lowest one, which suggests a relation between heat pre-treatment and the phenolic compound content. Through optical microscopy, microencapsulation led to better results with PGPR 4150, with visible polynuclear microcapsules. The particle size evaluation indicated that particles had a smaller size by number, but most of the core material was encapsulated in bigger particles. The mean diameter ranged from $20.29 \pm 0.85 \mu\text{m}$ to $24.00 \pm 1.27 \mu\text{m}$. High encapsulation efficiencies, determined by Folin-Ciocalteu's method, were obtained (99.71% to 99.81%).

The work provided relevant data on the valorisation and stabilisation of chestnut shell bioactive compounds, which are valuable in the food, cosmetic, and pharmaceutical industries.

Acknowledgements: This work is financially supported by the Promove Programme through Fundação “La Caixa”, in collaboration with the BPI and Fundação para a Ciência e a Tecnologia (FCT), under the project CyChest - Gestão integrada do ciclo do castanheiro no Parque Natural de Montesinho. Dr^a Isabel Martins, Dr^a Carina Costa and Professor Alírio Rodrigues, supervisors of this Dissertation, are members of LSRE-LCM – Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, supported by national funds through FCT/MCTES (PIDDAC): LSRE-LCM, UIDB/50020/2020 (DOI: 10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020); and ALiCE, LA/P/0045/2020 (DOI:10.54499/LA/P/0045/2020).

References:

- [1] Mateos-Aparicio and A. Matias, “Food industry processing by-products in foods,” in *The Role of Alternative and Innovative Food Ingredients and Products in Consumer Wellness*, C. M. Galaanakis, Ed. Academic Press, 2019, pp. 239–281.
- [2] E. Husanu et al., “Exploiting Deep Eutectic Solvents and Ionic Liquids for the Valorization of Chestnut Shell Waste,” *ACS Sustain. Chem. Eng.*, 8, (2020) 18386.
- [3] M. Faustino, M. Veiga, P. Sousa, E. M. Costa, S. Silva, and M. Pintado, “Agro-Food Byproducts as a New Source of Natural Food Additives,” *Molecules*, 24, (2019), 1.

Ohmic heating extraction of pectin from cacao shells: A sustainable and efficient approach for agroindustrial waste valorization

Jaqueline de Jesus Silva^{*}, Franciele Colussi², José teixeira², Marcelo Franco³

¹Department of Exact Sciences and Natural, State University of Southwest, Itapetinga, Bahia, Brazil

² Centre of Biological Engineering, University of Minho, Braga, Portugal

³Department of Exact Sciences and Technology, State University of Santa Cruz, Ilhéus, Bahia, Brazil

*2023m0236@uesb.edu.br

The depletion of natural resources threatens the sustainability of future generations, highlighting the importance of circular bioeconomy. In these models, waste is not discarded but incorporated into the production line, transforming into useful products. In this scenario, cacao presents itself as an opportunity, using the shells to extract pectin, a polysaccharide with various applications in the food, pharmaceutical and cosmetic industries. Less traditional methods such as ultrasound, microwave and subcritical fluid have already been reported in the extraction of pectin, but ohmic heating (OH), which is more energy efficient, has not yet been explored. It uses an electric field to raise the temperature of the sample uniformly, with low energy consumption and high efficiency, making it an environmentally friendly method. Based on this, the objective of this work was to extract pectin from the cacao shell by OH. For this, a mild treatment (20kHz, 2–7 V/cm, 60°C, 30min) was applied in an citric acid solution (pH 2), in a 1:20 ratio, which resulted in an average yield of 3%, similar to that obtained from cacao by Alava, et. al. (2021) with enzymatic hydrolysis, showing that the ohmic treatment under the applied conditions has an effect similar to that of the enzymatic complex used by them. Pereira et al. (2021) also observed similar results between the OH and conventional methods in the extraction of *Gracilaria vermiculophylla*, showing its potential to extract compounds with greater efficiency and lower energy expenditure, and it is recommended to test more severe conditions such as time and elevated temperatures. It is concluded, therefore, that OH is not only promising, but is also aligned with the principles of circular bioeconomy due to its energy efficiency and lower environmental impact, standing out for innovation and sustainability.

Acknowledgments: We are grateful for the financial support from Capes and CNPq and the technical support from Centre of Biological Engineering, University of Minho.

References:

- [1] Sara G. Pereira, Catarina Teixeira-Guedes, Gabriela Souza-Matos, Élia Maricato, Cláudia Nunes, Manuel A. Coimbra, José A. Teixeira, Ricardo N. Pereira, Cristina M.R. Rocha, Influence of ohmic heating in the composition of extracts from *Gracilaria vermiculophylla*, *Algal Research*, Volume 58, (2021).
- [2] Y J. R. Alava, J C. M. Moreira, S. I. M. Velez, D. M. P. Figueroa. Elevación de dos métodos de extracción de pectina de la cáscara de cacao (*Theobroma cacao*). *Revista Sinapsis*. Vol. 2, n° 20 (2021).
- [3] D. T. V. Pereira, P. Méndez-Albiñana, J. A. Mendiola, M. Villamiel, A. Cifuentes, J. Martínez, E. Ibáñez. An eco-friendly extraction method to obtain pectin from passion fruit rinds (*Passiflora edulis* sp.) using subcritical water and pressurized natural deep eutectic solvents, *Carbohydrate Polymers*, Volume 326 (2024).
- [4] J. C. G. Alencar, D. N. S. S. Batista, J. C. Souza, I. P. P. Santos, J. L. Bicas, M. E. O. Mamede, B. N. Paulino. Dual-objective optimization of ultrasound-assisted organic acid extraction of pectin from umbu (*Spondias tuberosa* L.): a promising Brazilian native fruit from Caatinga biome. *Biomass Conversion and Biorefinery*, (2024).

Exploring agro-industrial by-products for bioactive potential in food packaging: A study on *Punica granatum L.* and *Castanea sativa*

Joana C.L. Martins^{1,2,3*}, **Juliana Garcia**^{1,2,5}, **Rafaela Guimarães**^{1,5}, **Ana Cristina Gonçalves**⁵, **Maria José Saavedra**^{2,3,4}, **Maria José Alves**^{1,5,6,7}

¹AquaValor – Centro de Valorização e Transferência de Tecnologia da Água, Portugal;

²CITAB - Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Universidade de Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000801 Vila Real, Portugal

³CECAV - Veterinary and Animal Research Centre and Associate Laboratory for Animal and Veterinary Science (AL4Animals), University of Trás-os-Montes and Alto Douro, 5000-801 Vila Real, Portugal

⁴AB2Unit - Antimicrobials, Biocides & Biofilms Unit and Veterinary Sciences Department University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal

⁵LiveWell - Research Centre for Active Living & Wellbeing, Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal

⁶CIMO - Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal.

⁷Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

* martinsjoanacl@gmail.com

Environmental challenges associated to conventional plastic packaging have driven the search for ecofriendly alternatives [1]. The food industry, one of the largest consumers of plastic, also generates significant amounts of waste and by-products from various sources [2,3]. These agro-industrial residues, rich in bioactive compounds with potent antioxidant and antimicrobial properties, can be repurposed to extend the shelf life of food products, not only reducing dependence on synthetic additives but also mitigating the environmental impact [4,5].

The present study aimed to assess the bioactive potential of agro-industrial by-products, including *Punica granatum L.* peel, and *Castanea sativa* hedgehog, to evaluate the possibility of incorporating one of these extracts as a source of bioactive compounds in composite films. To prepare the crude extract, we employed a solid-liquid extraction process, followed by freeze-drying to obtain the dried extracts. A screening of the extracts was conducted to evaluate their antimicrobial activity using the Minimum Inhibitory Concentration (MIC) method against key pathogens, including *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. *Punica granatum L.* showed MIC values ranging from 1.95 mg/mL to 3.91 mg/mL, while the hedgehog extract of *Castanea sativa* showed MIC values between 0.250 mg/mL and 1.00mg/mL. Subsequently, the bestperforming extract was forward analysed. *Castanea sativa* hedgehog extract demonstrates significant antioxidant activity, with a Trolox Equivalent of Antioxidant Capacity (TEAC) value of $172,64 \pm 8,23$ (mg Trolox/ g) as determined by the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and $7,29 \pm 0,26$ (mg Trolox/ g) by Ferric Reduction Antioxidant Power (FRAP) assay. Overall, this study highlights the promising role of these agro-industrial by-products as a promising natural source of bioactive compounds for future applications in bioactive packaging materials.

Acknowledgments: This work is supported by National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UID/04033 And LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>). The authors are grateful to the Fundação “La Caixa” and to FCT for the financial support through Project “WoodlandFungi - Valorização e Sustentabilidade dos Cogumelos Silvestres do Barroso”, Projetos-Piloto Inovadores “Programa Promove - O futuro do Interior”, call 2023. R. Guimarães and J. Garcia acknowledge national public funding through “Investimento RE-C05-i02 – Missão Interface N.º 01/C05-i02/2022”, a project supported under the PRR (www.recuperarportugal.gov.pt). and financed by the European Union/Next Generation EU. Joana Martins is grateful to the –Portuguese Foundation for Science and Technology (FCT) for her PhD Grant (2023.05610.BDANA).

References:

- [1] N.L. Cristofoli, A.R. Lima, R.D.N. Tchoukouang, A.C. Quintino, M.C. Vieira, Sustainability, 15 (2023) 6153.
- [2] D. Pinto, M. Cádiz-Gurrea, A. Vallverdú-Queralt, C. Delerue-Matos, F. Rodrigues, Food Research International, 144 (2021) 110364.
- [3] M.F. Vargas-Torrico, M.A. Aguilar-Méndez, E. Ronquillo-de Jesús, M.R. Jaime-Fonseca, E. von BorriesMedrano, Food Hydrocolloids, 150 (2024) 109677
- [4] Thulasisingh, K. Kumar, B. Yamunadevi, N. Poojitha, S. SuhailMadharHanif, S. Kannaiyan, “Polymer Bulletin, (2022) 79.
- [5] Rzaeva, F. Coffigniez, N. Zeynalov, N. Gontard, V. Guillard, Frontiers in Nutrition., 10 (2023) 1223638.

Assessment of olive oil industry by-products as a source of phytoprotective agents for agriculture

Naiara Fernández^{1,*}, Frédéric B. Gaspar^{1,2}, Ana Luísa Oliveira¹, Mário Vale¹, Margarida Pimenta¹, Maria Teresa Barreto Crespo^{1,2}

¹IBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901, Oeiras, Portugal

² Instituto de Tecnologia Química e Biológica, António Xavier Universidade Nova de Lisboa, Av. da República, 2780-157, Oeiras, Portugal

*naiara.fernandez@ibet.pt

The European Green Deal and Farm to Fork strategy highlight the need to reduce synthetic pesticide use by 2030, addressing concerns about their persistence in the environment and associated risks to human health. In response, natural alternatives to synthetic pesticides are being explored. Olive oil industry by-products, such as olive pomace (OP) and olive leaves (OL), are abundant and low-cost sources of bioactive compounds. Besides presenting antioxidant activity (AA) [1,2], OP and OL extracts may also exhibit antimicrobial properties like other plant-based extracts [3,4], making them potential candidates for sustainable phytosanitary applications. This study is focused on extracting bioactive compounds from olive oil by-products and evaluating their potential to combat crop pests, acting as a biocompatible antioxidant and plant antifungal agent. As a first study, solid-liquid extraction was conducted using solvents of varying polarities at different temperatures. The efficiency of the extractions was evaluated, the AA of extracts was measured by the ORAC method, and its antifungal activity was then assessed *in vitro* against *Botrytis cinerea* and *Venturia pyrina* using an antimicrobial susceptibility testing method based on the antagonistic effect between the extract and the fungal targets. Regarding AA, OL extracts exhibited higher values than OP extracts, with maximum values of $119.20 \pm 4.33 \text{ mg}_{\text{GAE}}/\text{g}_{\text{extract}}$ for the ethanol Soxhlet OL extract. This value is comparable to those of commercial antioxidants like butylated hydroxytoluene (BHT) and α -tocopherol. In the antifungal assays, the extracts obtained from both by-products using apolar solvents exhibited activity against *Botrytis cinerea*. The most active extracts against *Venturia pyrina* came from OP and were obtained with different organic solvents, including ethanol and *n*-hexane, among others.

In conclusion, olive oil by-products are a promising source of phytosanitary ingredients. However, further research is needed to purify and concentrate bioactive compounds to enhance their effectiveness and explore sustainable alternatives against other pests.

Acknowledgments: TEC4GREEN Agenda, financed by the Portuguese Recovery and Resilience Plan (PRR). Authors also acknowledge FCT (Portugal) through R&D Units iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and GREEN-IT (UIDB/04551/2020, DOI: 10.54499/UIDB/04551/2020 and UIDP/04551/2020, DOI: 10.54499/UIDP/04551/2020) and LS4FUTURE Associated Laboratory (LA/P/0087/2020, DOI: 10.54499/LA/P/0087/2020).

References:

- [1] L. Schmidt, O.D. Prestes, P.R. Augusti, J.C. Fonseca Moreira, Phenolic compounds and contaminants in olive oil and pomace – A narrative review of their biological and toxic effects, *Food Biosci* 53 (2023) 102626. <https://doi.org/https://doi.org/10.1016/j.fbio.2023.102626>.
- [2] P. Otero, P. Garcia-Oliveira, M. Carpena, M. Barral-Martinez, F. Chamorro, J. Echave, P. Garcia-Perez, H. Cao, J. Xiao, J. Simal-Gandara, M.A. Prieto, Applications of by-products from the olive oil processing: Revalorization strategies based on target molecules and green extraction technologies, *Trends Food Sci Technol* 116 (2021) 1084–1104. <https://doi.org/https://doi.org/10.1016/j.tifs.2021.09.007>.
- [3] L.D.-Y.C.E.H.B.S.J.J. Lee Song Hee Oh Young Taek, Large-Scale Screening of the Plant Extracts for Antifungal Activity against the Plant Pathogenic Fungi, *Plant Pathol J* 38 (2022) 685–691. <https://doi.org/10.5423/PPJ.NT.07.2022.0098>.
- [4] Maurya, J. Prasad, S. Das, A.K. Dwivedy, Essential Oils and Their Application in Food Safety, *Front Sustain Food Syst* 5 (2021). <https://doi.org/10.3389/fsufs.2021.653420>.

Olive oil byproducts extracts: Bioactive potential in advanced cell models of intestinal inflammation and colorectal cancer

Melanie S. Matos^{1,2}, Joana Gonçalves^{1,2,3}, Carolina Ventura^{1,3}, Joana Marto⁴, Helena Ribeiro⁴, Elisabete Alexandre⁵, Naiara Fernández¹, Maria Rosário Bronze^{1,2,4}, Ana Teresa Serra^{1,2,*}

¹ iBET, Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal

² Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal

³ SOVENA Portugal Consumer Goods, S.A., Rua Dr. António Loureiro Borges, Edifício Arquiparque 2 Miraflares, 1495-131 Algés, Portugal

⁴ Research Institute for Medicines (iMed.Ulisboa), Faculdade de Farmácia da Universidade de Lisboa, Av Prof. Gama Pinto, 1649-003 Lisboa, Portugal

⁵ PERFINOX – Indústrias Metalúrgicas SA, Zona Industrial Lameiradas Lote 1, Mansores, 4540-423 Arouca, Portugal

*tserra@ibet.pt

The olive oil industry generates large amounts of byproducts, among which are olive pomace (OP) and leaves (OL) [1]. These matrices are rich sources of hydroxytyrosol and oleuropein, phenolic compounds with several health-promoting bioactivities [2]. This study aimed to evaluate the anti-inflammatory and anticancer potential of natural extracts obtained from OP and OL, thereby contributing to the valorization of these byproducts.

Given the critical role of chronic inflammation in tumorigenesis, advanced cell-based models representing intestinal inflammation and colorectal cancer were employed. The anti-inflammatory potential of the extracts was assessed using a physiologically relevant model of the inflamed intestinal mucosa, composed of enterocytes, goblet cells, and antigen-uptake facilitator microfold (M)-cells, in Transwell® devices. The antiproliferative effects of the extracts were assessed using different colorectal cancer cell lines: 1) HT29, derived from a primary tumor; and 2) LoVo, with metastatic characteristics. These cells were cultured in 3D models, which are more representative of the architecture of tumors. In the intestinal inflammation model, 0.5mg/mL OL extract was capable of significantly decreasing up to 32% the release of IL-8, a pro-inflammatory cytokine, into the cellular media. Conversely, both extracts could significantly prevent the loss of monolayer integrity (up to 99%) caused by the induced inflammatory stimulus, as measured by the trans-epithelial electrical resistance. These effects may be partially explained by the presence of hydroxytyrosol and oleuropein. The OP and OL extracts inhibited proliferation in both HT29 and LoVo, with EC₅₀ values of 1.11±0.22mg/mL and 1.45±0.13mg/mL, respectively. These effects could be attributed not only to the presence of hydroxytyrosol (EC₅₀=2.65±0.79mg/mL in HT29 and 2.24±0.02mg/mL in LoVo) but also to eventual additive or synergistic interactions among other phenolic compounds present in the extract.

Overall, the extracts derived from the olive oil industry byproducts are promising sources of bioactive compounds with anticancer and anti-inflammatory properties, with potential for application in nutraceutical formulations.

Acknowledgments: The authors acknowledge the PRR through the Agenda VIIAFOOD – Plataforma para a Valorização, Industrialização e Inovação comercial para o Agroalimentar”, framed in the notice nº 02/C05-i01/2022. The authors also acknowledge the funding from Fundação para a Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal) through iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020) and the Associate Laboratory LS4FUTURE (LA/P/0087/2020). Teresa Serra also acknowledges FCT/MCTES for her individual grand (CEECIND/04801/2017).

References:

- [1] S. Selim, M. Albqmi, M.M. Al-Sanea, *et al.*, *Front. Nutr.*, 9 (2022).
- [2] F. Gervasi, F. Pojero, *Biomedicines*, 12 (2024).

***In Vitro* and *In planta* screening of bacterial control agents against Pear Scab with Imaging and molecular detection methods**

**Sara Tedesco^{1,2*}, João P. Baixinho^{1,2}, Margarida Pimenta^{1,2}, Filipa T. Silva^{1,2},
Frédéric B. Gaspar^{1,2}, Francisco X. Nascimento^{1,2}, Teresa Crespo^{1,2}**

¹ iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

² ITQB, Instituto de Tecnologia Química e Biológica António Xavier Universidade Nova de Lisboa, Oeiras, Portugal

* sara.tedesco@ibet.pt

The Rocha pear (*Pyrus communis* L. cv 'Rocha') is of significant economic value in Portugal, particularly in the Oeste region, where it holds Protected Designation of Origin status and is one of the country's most important fruit export [1]. However, its cultivation faces a serious threat from European pear scab caused by the fungus *Venturia pyrina*, which severely impacts both yield and fruit quality [2]. Traditional management of this disease relies heavily on chemical fungicides, but the European Green Deal's goal of reducing pesticide use by 50% by 2030 underscores the urgent need for sustainable alternatives [3].

Bacteria with antimicrobial properties present a promising solution for controlling pear scab, as their vast diversity offers significant potential for biopesticide development [4]. However, efficient screening methods are crucial, as field evaluations are time-consuming and dependent on natural infection cycles, making them impractical for rapid screening. *In vitro* inhibition assays and detached leaf methods have been widely used to assess the efficacy of biological control agents against various pathogens [5], but their adaptation for *V. pyrina* remains underexplored.

This study introduces optimized *in vitro* methods for evaluating and quantifying the inhibitory activity of bacterial strains against *V. pyrina*. To further assess bacterial effectiveness *in planta*, we developed a detached leaf assay using Rocha pear leaves, which enables the examination of interactions between bacteria, the pathogen, and the host plant. Additionally, two complementary quantification methods were established: an imaging-based approach with ImageJ to measure lesion severity and a real-time qPCR assay for precise molecular quantification of *V. pyrina* DNA in pear leaves.

These tools enabled us to correctly detect and quantify effective bacterial strains with antifungal properties, contributing to the development of sustainable pear scab management strategies for Rocha pear cultivation while aligning with the European Green Deal's objectives.

Acknowledgments: TEC4GREEN Agenda, financed by the Portuguese Recovery and Resilience Plan (PRR). Authors also acknowledge FCT (Portugal) through R&D Units iNOVA4Health – UIDB/04462/2020 and UIDP/04462/2020, GREEN-IT Bioresources for Sustainability Unit – UIDB/04551/2020, DOI: 10.54499/UIDB/04551/2020 and UIDP/04551/2020, DOI: 10.54499/UIDP/04551/2020, and LS4FUTURE Associated Laboratory - LA/P/0087/2020, DOI: 10.54499/LA/P/0087/2020.

References:

- [1] X. Lomba-Viana, A. Raymundo, C. Prista, M.J. Alegria, I. Sousa, *Frontiers in Nutrition*, 9 (2022) 825999.
- [2] G. Lăcis, I. Kota-Dombrovska, B. Lăce, *Acta Horticulturae*, 1327 (2021) 57.
- [3] Wesseler, J., *Applied Economic Perspectives and Policy*, 44(4) (2022) 1826.
- [4] A. Bonaterra, E. Badosa, N. Daranas, J. Francés, G. Roselló, E. Montesinos, *Microorganisms*, 10(9) (2022) 1759.
- [5] F. Daayf, L. Adam, W.G.D. Fernando, *Canadian Journal of Plant Pathology*, 25(3) (2003) 276.

Biocontrol potential of novel rhizospheric bacterial strains against *botrytis cinerea*: Functional validation and genomic insights

Sara Tedesco^{1,2*}, Filipa T. Silva^{1,2}, Margarida Pimenta^{1,2}, Frédéric B. Gaspar^{1,2},
Francisco X. Nascimento^{1,2}, Teresa Crespo^{1,2}

¹ iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

² ITQB, Instituto de Tecnologia Química e Biológica António Xavier Universidade Nova de Lisboa, Oeiras, Portugal

*sara.tedesco@ibet.pt

Among the most pressing challenges in crop protection is the control of *Botrytis cinerea*, the pathogen responsible for gray mold, which threatens a broad range of economically valuable crops worldwide [1]. Although several chemical fungicidal compounds are currently used to control *B. cinerea*, the rising demand for sustainable agricultural practices has intensified the need for biological alternatives [2].

In this work, we demonstrate the potential of two novel bacterial isolates, *Pseudomonas* sp. NFX104 and *Serratia* sp. NFX21, obtained from Portuguese natural rhizosphere/soil samples, to inhibit the growth of *B. cinerea*. *In vitro* dual-culture plate assay demonstrated that both strains produced compounds that significantly inhibited the growth of *B. cinerea* with activity comparable to Serenade®, a commercial biopesticide based on a *Bacillus* strain [3]. Moreover, detached tomato leaf assays (cv. Money Maker) showed that the application of the bacterial strains led to decreased *B. cinerea* pathogenicity, with lesion reductions of 73.1% (NFX104) and 63.7% (NFX21), comparable to that of a commercial solution (Serenade®, 68.80% reduction).

Genomic analysis revealed the presence of several biosynthetic gene clusters involved in the production of antifungal compounds in the bacterial strain's genomes. Strain NFX104 harbored clusters for 2,4-diacetylphloroglucinol and hydrogen cyanide production, which are known to have antifungal properties [4,5]. Strain NFX21 genome contained several chitinase genes and a cluster involved in pyrrolnitrin biosynthesis, involved in fungal antagonism [6,7].

Ultimately, the obtained results underscore the potential of NFX104 and NFX21 as effective biological control agents against gray mold. Their ability to produce diverse antifungal compounds, combined with their proven efficacy in controlling *B. cinerea* both *in vitro* and *in planta*, positions them as valuable tools in the development of sustainable crop protection strategies contributing to the transition toward more sustainable agricultural practices.

Acknowledgments: TEC4GREEN Agenda, financed by the Portuguese Recovery and Resilience Plan (PRR). Authors also acknowledge FCT (Portugal) through R&D Units iNOVA4Health – UIDB/04462/2020 and UIDP/04462/2020, GREEN-IT Bioresources for Sustainability Unit – UIDB/04551/2020, DOI: 10.54499/UIDB/04551/2020 and UIDP/04551/2020, DOI: 10.54499/UIDP/04551/2020, and LS4FUTURE Associated Laboratory - LA/P/0087/2020, DOI: 10.54499/LA/P/0087/2020.

References:

- [1] N. Cheung, L. Tian, X. Liu, and X. Li, *Pathogens*, 9(11) (2020) 923.
- [2] Wesseler, J., *Applied Economic Perspectives and Policy*, 44(4) (2022) 1826.
- [3] R. Lahlali, G. Peng, B. Gossen, L. McGregor, F. Yu, R. Hynes, S. Hwang, M. McDonald, S. Boyetchko, *Phytopathology*, 103(3) (2013) 245.
- [4] E.T. Johnson, M.J. Bowman, R.P. Gomes, L.C. Carneiro, C.A. Dunlap, *Scientific Reports*, 13(1) (2023) 14292.
- [5] Sehrawat, S.S. Sindhu, B.R. Glick, *Pedosphere*, 32(1) (2022)15.
- [6] G. Singh, S.K. Arya, *Biocatalysis and Agricultural Biotechnology*, 20 (2019) 101289.
- [7] S. Pawar, A. Chaudhari, R. Prabha, R. Shukla, D.P. Singh, *Biomolecules*, 9(9) (2019) 443.

Valorisation of wine by-products: Extraction and characterization of antioxidants for food applications

Manuela M. Moreira^{1,*}, Cristina Soares¹, Cristina Delerue-Matos

¹REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto,
Rua Dr. António Bernardino de Almeida, 431, 4249-015, Porto, Portugal

* mmdsm@isep.ipp.pt

The winemaking sector is one of the most important worldwide, which translates into the production of huge amounts of by-products, such as grape pomace and stalks, with high environmental impacts [1]. These by-products represent potential sources of natural polyphenols, and due to their recognized health-promoting properties, several studies have focused their efforts on their efficient extraction [2].

This study aims to determine the potential of the wine by-products as source of antioxidants for their potential incorporation in food products. For that, three environmental-friendly extraction techniques, namely ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and subcritical water extraction (SWE), were tested and compared with a conventional extraction (CE) using grape by-products from two varieties (*Tinta Miúda* and *Cerceal Branco*). The extracts were assessed for total phenolic content (TPC), total tannin content (TTC), and antioxidant/antiradical activities (FRAP and ABTS assays).

Among the tested methods, SWE enables us to recover the highest amount of polyphenols. The *Cerceal Branco* grape stalk extract obtained via SWE at 150 °C exhibited the highest TPC (17.0 ± 0.2 mg GAE/g fresh weight (fw)), while the *Tinta Miúda* grape stalk extract showed the strongest antioxidant activity (FRAP = 15.9 ± 0.3 mg AAE/g fw; ABTS = 20.9 ± 1.0 mg AAE/g fw). Ongoing research focuses on identifying the specific phenolic compounds responsible for the exhibited properties using high-performance liquid chromatography with diode array detection.

These findings demonstrate that SWE is a highly efficient and sustainable technique for extracting bioactive compounds from grape by-products. By integrating such green technologies into the food industries, this research contributes to circular economy strategies, transforming winemaking by-products into high-value functional ingredients.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Manuela M. Moreira (2023.05993.CEECIND/CP2842/CT0009) is thankful for her contract financed by FCT/MCTES—CEEC Individual Program Contract and to REQUIMTE/ LAQV. The supply of grapevine by-products is acknowledged to AVIPE.

References:

- [1] C. Ferreira, M. Moreira, C. Delerue-Matos, and M. Sarraguça. *Molecules*, 28 (2023), 7538.
- [2] E. Tapia, A. Silva, C. Delerue-Matos, M. Moreira, F. Rodrigues, R. Torres Carro, M. Santi, M. Ortega, M. Blázquez, M. Arena, and M. Alberto. *Foods*, 13 (2024), 1795.

Cork extracts as bioactive agents: Antioxidant potential and formulation strategies

Nuno Silva¹, Ana Colette Maurício², Ruben Fernandes³ and Ana Novo Barros⁴

¹ULSSA, Porto, Portugal

²CECA-ICETA/AL4AnimalS/ICBAS-UP, Porto, Portugal

³CECLIN/UFP, Gondomar, Portugal

⁴ Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Universidade Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal
nuno.malafaia@hotmail.com

The bioactive potential of cork extracts has gained increasing attention due to their remarkable antioxidant and anti-inflammatory properties. This study aims to evaluate the therapeutic potential of cork-based formulations by analyzing the effects of different concentrations and combinations of cork extracts on anti-aging properties and overall product efficacy. Various formulation strategies were explored to maximize the bioactivity of cork extracts, particularly their ability to combat oxidative stress and inflammation. To achieve this, six colorimetric assays were conducted to assess the antioxidant capacity of cork extracts, providing a comprehensive evaluation of their bioactive properties. The results revealed a strong antioxidant profile across all assays, indicating a high concentration of bioactive compounds with free radical-scavenging properties. The presence of polyphenolic compounds and other antioxidative agents was confirmed, demonstrating their ability to mitigate oxidative stress. Additionally, the data showed a concentration-dependent effect, where higher extract concentrations correlated with enhanced antioxidant activity. The study also highlighted the influence of different extract combinations on overall efficacy, emphasizing the importance of formulation strategies in optimizing cork's bioactive potential. These findings support the application of cork extracts in cellular protection, particularly in formulations designed to counteract oxidative damage and inflammation-related aging processes. The results underscore the significant antioxidant properties of cork extracts, making them promising candidates for use in cosmetic and biomedical applications. Given their potential to reduce oxidative stress, further research should focus on optimizing extraction methods and formulation techniques to enhance their stability, bioavailability, and therapeutic effectiveness.

Keywords: Cork, Antioxidant, Inflammation, Bioactive Compounds, Anti-aging

INNOECOFOOD – Eco-Innovative technologies for improved nutrition, sustainable production and marketing of agroecological food products in Africa

Mariana Palma^{1*}, Marta Dias¹, Alícia Pereira¹, Sara Moutinho¹, Cheila Almeida^{1,2}, Amparo Gonçalves^{1,2}, Carla Pires^{1,2}, Luísa M. P. Valente^{1,3}, Leonor Nunes¹, António Marques^{1,2}

¹ CIIMAR - Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal

² IPMA - Portuguese Institute for the Sea and Atmosphere, Algés, Portugal

³ ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

* mpalma@ciimar.up.pt

The INNOECOFOOD project is designed to develop and promote eco-innovative strategies to improve the sustainable production and marketing of agro-ecological products with high nutritional value in Africa. This will be achieved by pioneering production/business ECOHUBs and boosting local aquaculture farms to innovatively develop the value chains of catfish and tilapia, spirulina and insects to produce certified marketable food and feed products and ingredients, based on the "farm to fork" approach. INNOECOFOOD aims to 1) build four sustainable and innovative rural agribusiness ECOHUBs, using renewable energy for production, processing and marketing activities to address the AU and EU green deal and trade; 2) optimize the production of ethical, eco-farmed fish, spirulina and insects, sustainable feeds and post-harvest processing technologies using sensors, AI and IoT technology; 3) scope suitable vegetable products/ingredients and up cycle fish, spirulina and insect coproducts towards novel products by solar powered drying and extrusion processing, using AI sensors and IoT; 4) validate the nutritional and sensory quality, microbiological and chemical safety, consumer acceptance, value environmental impacts with Life Cycle Assessment (LCA), and implement certification schemes for fish, spirulina and insect based products/ingredients targeting marketing and consumption; 5) create a database and locally train 120 young people and 120 women in the ECOHUBs and 5000 rural workers.

Among the activities carried out by CIIMAR are 1) production of tilapia to assess the impact of a feed formulated with local raw materials (in Portugal) mimicking ingredients available in partner countries, compared to a commercial diet; 2) optimisation of a strategy for upcycling seafood by-products to produce protein hydrolysates with good biological activities; 3) collaboration in the drafting of two technological and analytical procedure manuals and 4) definition of an appropriate LCA strategy to assess the environmental impact of the production processes of manufactured products.

Acknowledgments: This is a project financed by Horizon Europe and UK Research and Innovation, and it comprises an experienced multi-actor consortium of 20 partners in Europe and Africa and rural communities.

NEWPACK - A New Generation of Packaging Materials

Sofia Lourenço^{*1}, Luis Aparicio¹, Paula Allen¹, Ana Luísa Maulvault², António Marques², João C. Bordado³, Rui Galhano dos Santos³, Ana C. Marques³, Márcia Damas⁴, Pedro Santana⁴

¹ Sovena Portugal Consumer Goods, S.A., Rua Dr. António Loureiro Borges nº 2, Edifício Arquiparque 2, 3º andar, 1495-131 Algés, Portugal

² Instituto Português do Mar e da Atmosfera (IPMA I.P.), Divisão de Aquacultura, Av. Doutor Alfredo Magalhães Ramalho 6, 1495-165, Lisboa, Portugal

³CERENA, DEQ – Instituto Superior Técnico, Av. Rovisco Pais, no. 1, 1049-001, Lisboa, Portugal

⁴Logoplaste Innovation Lab (ILAB), Estrada da Malveira 900 2750-834 Cascais, Portugal
** sofia.lourenco@sovena.pt*

Currently, Polyethylene Terephthalate (PET) stands as one of the most broadly utilized polymers in food packaging. However, recent years have witnessed significant research efforts aimed at developing alternative materials capable of replacing PET and other petroleum-based plastics in food-related applications. In this regard, biopolymers derived from renewable raw materials are considered particularly promising alternatives. When biodegradable they further support the transition to a more sustainable, and green economy, while their durable properties, such as resistance to chemical and enzymatic reactions, enhance their applicability in diverse contexts. Within this framework, the NEWPACK project aims to develop a new generation of packaging materials by integrating the following innovations: (I) the use of renewable raw materials as alternatives to PET, with compositions that, in the event of accidental release into the marine environment, pose no harm to aquatic life and may even have a nutritional effect upon ingestion; (II) the incorporation of active packaging systems formulated with natural ingredients to optimize the shelf life of food products; and (III) the implementation of intelligent packaging equipped with sensors capable of providing real-time information on product storage conditions and enabling tracking throughout the supply chain, thereby reducing food waste.

This groundbreaking project has undergone several key phases, including the selection of monomers and the formulation of polymers through comprehensive *in vitro* and *in vivo* testing with marine fish, specifically gilthead seabream (*Sparus aurata*). These tests aimed to evaluate the ecotoxicological and physiological responses of the fish to candidate monomers, determining their toxicity levels through ingestion, assessing their lethal concentrations for aquatic organisms, and selecting monomers and corresponding lethal doses for polymer synthesis.

The design of this novel packaging material represents a significant advancement in reducing the carbon footprint associated with packaging systems. Furthermore, it paves the way for innovative circular economy approaches, particularly in the context of food packaging applications, including edible oil products.

Acknowledgments: This research was supported by the NextGenerationEU programme under the Recovery and Resilience Facility Plan for Portugal - Agenda VIIAFOOD - Platform for Valorization, Industrialization, and Commercial Innovation for the Agri-Food Sector (no. C644929456-00000040)



Organizational Life Cycle Assessment of innovation centre for agri food industry: Evaluating the carbon footprint of a colab

Talha Anwar^{1,*}, André Mota¹, Daniel Sousa¹, Dalila Vieira¹

¹ Colab4Food - Collaborative Laboratory for Innovation in the Agri-Food sector, Portugal

* *talha.anwar@colab4food.com*

This study presents an Organizational Life Cycle Assessment (O-LCA) of Colab4Food, a research and development (R&D) company specializing in sustainability assessment, ingredient research, regulatory services, market analysis, and food databases for the agrifood industry. Unlike traditional LCAs focused on products, an O-LCA evaluates the environmental footprint of an entire organization, offering insights into the carbon emissions associated with daily operations and tasks. The primary objective of this study is to quantify Colab4Food's carbon footprint and develop a replicable framework for assessing the environmental impact of research collaborations.

The study adopts an O-LCA approach aligned with ISO 14072 [1], encompassing direct and indirect emissions from operational activities. Key impact sources include energy consumption, employee commuting, business travel, office consumables (e.g., printing paper), and water usage. The reporting unit for the assessment was 1st Quarter of 2024 work done by 22 employees. Data collection was based on activity records, utility bills, and employee surveys, with emissions quantified using emission factors from recognized databases like Ecoinvent [2]. Preliminary results indicate that Colab4Food's operations generate approximately 1.04 tons of CO₂-equivalent emissions per employee over 1st Quarter of 2024. The largest contributions come from business travel and employee commuting, highlighting mobility as a critical area for emission reduction.

This assessment not only quantifies the carbon footprint of an R&D-focused organization but also provides a methodological framework for conducting O-LCAs in similar research collaborations. The framework integrates primary and secondary data sources, ensuring a comprehensive evaluation of an organization's environmental impact. Insights from this study can guide sustainability strategies for Colab4Food, such as optimizing remote work policies, reducing non-essential travel, opting for public transportation and carpooling among employees improving resource efficiency. Ultimately, this research demonstrates how research and development organizations can systematically assess and reduce their environmental footprint.

Acknowledgments: This work was supported by the Missão Interface funding programme. The authors acknowledge the financial support provided, which has been instrumental in carrying out this research.

References:

- [1] "ISO 14072," 2024. [Online]. Available: <https://www.iso.org/standard/86265.html>.
- [2] ecoinvent, "Ecoinvent," 2024. [Online]. Available: <https://ecoinvent.org/>.



An industrial waste stream to a final product: The valorisation and incorporation of a tomato ketchup by-product

Ana Catarina Costa^{1,*}, Maria Ramos², Ana Tasso², Anabela Raymundo¹, Catarina Prista¹

¹Instituto Superior de Agronomia, LEAF (Linking Landscape Environment Agriculture and Food) Research Center, Tapada da Ajuda, 1349-017 Lisboa, Portugal

² Mendes Gonçalves, Zona Industrial, lote 6, Golegã, 2154-909 Golegã, Portugal

* acatarinacosta@isa.ulisboa.pt

In Portugal, Casa Mendes Gonçalves is a reference company in the production of sauces and condiments. In 2023, 18,928 tonnes of sauces and condiments were produced, and only 0.5% were valorised for reutilisation [1]. Industrial sauce operations generate waste streams with interesting characteristics, such as high nitrogen and carbon content, making them attractive for upcycling through fermentation [2].

This work aimed to produce and characterise a fermented ketchup stream (KCFFM) to be incorporated into the target product. The first step was to ferment the ketchup waste stream (containing sorbate) at a laboratory scale. Stream samples were diluted 1:4 (25% incorporation) with demineralised water, inoculated with a consortium of three lactic acid bacteria and one yeast, and allowed to ferment at room temperature for 7 days. During fermentation, samples were collected, and pH, TSS and titratable acidity were analysed. Metabolites produced by the consortium were evaluated at the end of the fermentation.

The KCFFM's pH at the beginning of fermentation was 3.35 and ended at 2.80, while the TSS values show glucose consumption through a decrease in °Brix, from 9.20 to 7.50 °Brix. According to the consortium's fermentative metabolism, the titratable acidity at the end of the fermentation reached 1.25g/100g of fresh biomass, which is consistent with the increase in the concentration of organic acids throughout the fermentation, reaching lactic acid values of 1.34g/100g of fresh biomass.

In a second phase, pilot-scale fermentations were carried out by Casa Mendes Gonçalves and used in a new formulation of the target ketchup containing 40% of the fermented product. Nutritional and physical analyses of the new product were performed to evaluate the impact of the fermented stream on the developed prototype. Shelf life was determined through drop tests. Comparing the target to the reformulated prototype showed promising results due to the similarity to the target.

Acknowledgments: This work was financially supported by national funds from Agenda VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (nº C644929456-00000040), WP7, A7.3. Valorisation of waste streams for re-incorporation in sauces and UIDB/AGR/04129/2020 LEAF Research Unit.

References:

[1] Relatório Anual da Casa Mendes Gonçalves 2023: Criamos o futuro da alimentação, Golegã, 2024. <https://www.casamg.pt/sustainability/>.

[2] D.J. Faria, A.P.A. de Carvalho, C.A. Conte-Junior, Valorization of Fermented Food Wastes and Byproducts: Bioactive and Valuable Compounds, Bioproduct Synthesis, and Applications, Fermentation 9 (2023) 920. <https://doi.org/10.3390/fermentation9100920>.

Up-cycling broccoli leaves: lactic acid fermentation as a sustainable approach to reduce food loss

Tiago Miranda¹, Sara Simões², Anabela Raymundo², Mariana Mota², Catarina Prista^{2,*}

¹Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisboa, Portugal

² LEAF – Linking Landscape, Environment, Agriculture and Food Research Centre, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017, Lisboa, Portugal

* cpista@isa.ulisboa.pt

Broccoli (*Brassica oleracea* var. *italica*) has acquired considerable relevance in the last few years as a health-promoting food [1]. Broccoli stalks (softer/upper part) and florets, plant parts with commercial interest, are a small part of the edible total aerial biomass of the plant (~15 %). The potential consumption of stalks (harder/lower part) and leaves would increase productivity and sustainability of the broccoli value-chain by increasing yield from 15 % up to as much as 83 % [2]. In this project, fermentation was used to up-cycle broccoli co-products, namely leaves, to develop and optimize a new fermented food ingredient. Lactic acid bacteria (LAB) and yeast consortiums were tested as inoculum for broccoli leaves fermentation. The natural microbiota was isolated and identified by DNA sequencing, and LAB and yeasts were selected for their potential as inocula. Selected strains were obtained commercially and previously tested using drop-test methodology [3]. For the fermentation assay, combinations of five LAB (both homo and heterolactic) and three yeasts were tested in consortia. Different sodium chloride concentrations were tested to enhance LAB activity and limit potential contaminations (0.3, 0.5 and 1 % w/w). Furthermore, the initial pH value was adjusted to 4.5 using acetic acid (10 % w/w). Fermentation pH was monitored daily. The best fermentation conditions (consortium and NaCl) were selected considering the ability to decrease pH rapidly, as well as the production of organic acids, total pH decrease and preliminary sensory analysis. The fermentations reached pH values under 3.5, a good indicator of food safety. The best results (significant, $p < 0.05$) for pH value decrease after 24h of fermentation were obtained when *Weissella confusa*, an autochthonous bacteria present in the broccoli co-product, was part of the fermentation consortium. After upscaling assays, broccoli leaves could be a potential sustainable ingredient for the food industry.

Acknowledgements: This work was supported by *Fundação para a Ciência e a Tecnologia* (FCT) through the strategic project UID/AGR/04129/2020 (LEAF) and the PhD grant to Sara Simões (2022.10963.BD).

References:

- [1] A. M. Ares, M. J. Nozal, J. Bernal, *Journal of Chromatography A*, 1313 (2013)
- [2] Mengpei Liu, Lihua Zhang, Suk Lan Ser, Jonathan R. Cumming, Kang-Mo Ku, *Molecules*, 23 (2018) 4.
- [3] S. Simões, C. C. Sanchez, A. J. Santos, D. Figueira, C. Prista, A. Raymundo, *Foods*, 12 (2023) 7.

Enzymatic modelling of juice production pomace for novel ingredient development in non-dairy infant nutrition

**Ana Abraão^{1*}, Sofia Vieira², Maria Arranca³, Irene Fraga¹, Maria João Alegria⁴,
Luís Ferreira¹, Elisabete Coelho³, Ana Novo Barros¹**

¹ Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB,

² Inov4Agro, Universidade de Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

University of Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal 3
Department of Chemistry, University of Aveiro (UA), 3810-193 Aveiro, Portugal 4 SUMOL + COMPAL Marcas S.A., 2780-179 Carnaxide, Portugal.

* aabraao@utad.pt

Food waste management remains a critical global challenge, with its impact continuing to increase [1]. The juice industry, in particular, faces substantial issues related to the large quantities of by-products generated during fruit processing [2].

During the industrial processing of apple juice, a solid residue known as apple pomace is generated. Although this by-product contains a high concentration of essential nutrients and bioactive compounds, it is frequently discarded, resulting in both economic losses and environmental challenge [3]. To mitigate waste and enhance its value, this study aimed to develop children's cookies enriched with enzymatically treated apple pomace using Rapidase Fiber.

This study was developed in collaboration with SUMOL + COMPAL company and assessed the phenolic profile, antioxidant capacity, and nutritional composition of apple pomace flour before and after enzymatic treatment. Cookies were formulated with three levels of pomace incorporation: 0% (control), 11% hydrolyzed apple pomace, and 11% non-hydrolyzed apple pomace, followed by sensory analysis. The findings demonstrated that enzymatic treatment altered the dietary fiber composition, increasing soluble polysaccharides and enhancing the phenolic compound content in the hydrolyzed pomace sample, which was 19.6% higher than in its non-hydrolyzed counterpart. This modification contributed to an improvement in antioxidant capacity. Enzymatically treated apple pomace exhibited significantly lower ash content, while protein and fat levels remained largely unchanged, except for pomace sample 1, where fat content decreased post-hydrolysis. Cookies enriched with both hydrolyzed and non-hydrolyzed apple pomace received higher sensory scores than the control, particularly in terms of sweetness, overall acceptability, and purchase intention. These findings suggest that enzymatic treatment of apple pomace represents a promising strategy for its incorporation into children's food products and the development of innovative functional foods.

Acknowledgments: The authors acknowledge the support of the project VIIAFOOD - Platform for the Agri-Food valorisation, industrialization and Commercial Innovation. Reference C64492945600000040. This work is supported by National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UID/04033 And LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>). WASTELESS funding from HORIZONRIA (HORIZON-CL6-2022-FARM2FORK-01) under Grant Agreement No. 101084222

References:

- [1] Coelho, M.C.; Ghalamara, S.; Campos, D.; Ribeiro, T.B.; Pereira, R.; Rodrigues, A.S.; Teixeira, J.A.; Pintado, M. *Foods*, 12 (2023) 818.
- [2] Ignatia, F.; Meivira, K.; Kartawiria, I.S.; Gunawan-Puteri, M.D.P.T. Review: Nutrient, Fiber, and Bioactive Content of Fruit Pomace, Major By-Product of Juice Industry.; 2022.
- [3] Saba, B.; Bharathidasan, A.K.; Ezeji, T.C.; Cornish, K. Characterization and Potential Valorization of Industrial Food Processing Wastes. *Science of The Total Environment*, 868 (2023) 161550.

Winery by-products as a source of bioactive phenolics: antioxidant and enzymatic inhibitory properties

Irene Gouvinhas^{1,*}, Rui Dias Costa¹, Raúl Domínguez-Perles², Ana Novo Barros¹

¹ Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB)/Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro), University of Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal

² Phytochemistry and Healthy Foods Lab (LabFAS), CEBAS-CSIC (Consejo Superior de Investigaciones Científicas), Campus Universitario de Espinardo, Edif. 25, 30100 Murcia, Spain.

* igouvinhas@utad.pt

It is well established in the scientific community that agro-food by-products represent economic advantages and contribute to circular economy [1]. For instance, wine industries of *Região Demarcada do Douro* involve the production of large quantities of by-products [2], such as grape stems (GSt), grape pomace (GP), grape vine shoots (GVS), grape seeds (GSe), or wine lees (WL), presenting a remarkable valuable composition in phytochemicals, such as phenolic compounds [3], with putative health-promoting qualities, including anti-aging, cardioprotective, antibacterial, anti-inflammatory, and anticancer properties [4].

The objective of this study was to explore the phenolic composition and bioactive properties of different winery by-products (WBPs) from white and red grape varieties of the Douro Demarcated Region. To accomplish this aim, several methodologies were employed, including the assessment of total phenolic content (TPC), ortho-diphenols content (ODC), flavonoids content (FC), enzymatic inhibitory activity through tyrosinase and elastase inhibition assays, and antioxidant capacity determination using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assays. A solid-liquid extraction method employing a mixture of ethanol and water (50:50, v/v) was utilized to extract phenolic compounds from the WBPs. TPC exhibited a significant positive correlation with FC ($r=0.837$) and FRAP ($r=0.952$), as well as between FC and FRAP ($r=0.909$). Additionally, ODC displayed significant positive correlations with DPPH ($r=0.946$) and ABTS ($r=0.898$). The identification of phenolic compounds presented in GSt by HPLC-DAD was performed. Among the various WBPs investigated, GSt and GSe (Viosinho, Malvasia Fina, and Fernão Pires) demonstrated the highest values of phenolic content and antioxidant capacity, compared to other WBPs. Furthermore, the GSe (Viosinho, Malvasia Fina, and Fernão Pires) displayed the highest values of anti-elastase and anti-tyrosinase activities. This study highlights the potential of WBPs as valuable sources of bioactive compounds with antioxidant and enzymatic inhibitory properties, reinforcing their applicability in nutraceutical and cosmetic formulations.

Acknowledgments: Irene Gouvinhas thanks FCT (Fundação para a Ciência e Tecnologia) for funding through the Scientific Employment Stimulus—Individual Call (2022.00498.CEECIND), <https://doi.org/10.54499/2022.00498.CEECIND/CP1749/CT0001> (accessed on 07 February 2025). Rui Dias Costa acknowledges the financial support provided by National Funds by FCT - Portuguese Foundation for Science and Technology (FCT) PhD grant: 2021.07571.BD. This work is also supported by National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UI/04033 and LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>). We also acknowledge Rozés, Adega de Vila Real, and Daniel Fernandes – Unipessoal de Viticultura for the samples provided.

References:

- [1] I. Gouvinhas, M. Queiroz, M. Rodrigues, A.I.R.N.A. Barros, In *Polyphenols in Plants*, Elsevier, 2019; pp. 381–394 ISBN 9780128137680. A. Novo de Barros, Gouvinhas, I Eds.; IntechOpen, 2021; ISBN 978-1-83880-682-8.
- [2] F. J. Barba, Z. Zhu, M. Koubaa, A. S. Sant'Ana and V. Orlien, *Trends in Food Science and Technology*, 49 (2016) 96–109.
- [3] P. S. Melo, A. P. Massarioli, C. Denny, L. F. Dos Santos, M. Franchin, G. E. Pereira, T. M. F. D. S. Vieira, P. L. Rosalen and S. M. De Alencar, *Food Chemistry*, 181 (2015) 160–169.
- [4] K. Chakka and A. S. Babu, *Applied Food Research*, 2(2022) 100058.

Unlocking value of marine co-products through fish protein hydrolysates

**Carolina Camacho^{1,*}, Maria Sapatinha^{2,3}, Helena Oliveira^{1,2}, A. Rita Ribeiro⁴,
M. Leonor Nunes¹, António Marques^{1,2}, Carla Pires^{1,2}**

¹CIIMAR/CIMAR-LA - Interdisciplinary Centre of Marine and Environmental Research,
University of Porto, Matosinhos, Portugal

²IPMA, IP - Portuguese Institute for the Sea and Atmosphere, Division of Aquaculture,
Upgrading and Bioprospection, Algés, Portugal

³Nova School of Science and Technology, Nova University Lisbon, Department of Chemistry,
Caparica, Portugal.

⁴Blue Bioeconomy CoLAB, Leça da Palmeira, Portugal

*ccamacho@ciimar.up.pt

Every year, millions of tonnes of fish are wasted globally, and Portugal, one of the highest consumers of fish per capita, is particularly affected. The fish processing industry generates large amounts of co-products (20-80% of fish weight), which, within a circular economy and zero-waste framework, can be reused as valuable resources. In response to this challenge, the *Fish Matter* project was launched to create a platform connecting co-products generators with companies specialised in their transformation into added-value raw materials [1]. Among the promising solutions, fish protein hydrolysates (FPH) have emerged as an approach for converting co-products into value-added products.

This study explores the potential of FPH production from five Portuguese fish processing co-products: heads and bones of black scabbardfish (BS; ArtesanalPesca, C.R.L., Sesimbra) and gilthead seabream (Vivid Foods Lda., Atalaia), trimmings from hake and Nile perch (Gelpixe, S.A., Loures), and salmon heads (Auchan S.A.). Enzymatic hydrolysis was performed using food-grade endo- and exopeptidases in both single- and two-stage processes. The chemical composition, mineral profile, fatty acid content, and metal concentrations of co-products and dried FPH were analysed. Additionally, the hydrolysis yields as well as biological properties of FPH were also determined.

All FPH generally followed the chemical profile of their respective co-products, showing a rich protein and mineral composition with minimal metal content, except for BS-derived FPH. Single-stage hydrolysis with two enzymes yielded the highest protein recovery [2] but resulted in higher fat content compared to hydrolysis with a single enzyme. The findings highlight the enzymatic hydrolysis as an effective valorisation strategy, producing bioactive FPH with antioxidant and antihypertensive properties [2,3]. This approach not only enhances the nutritional and economic value of fish co-products but also contributes to waste reduction and the development of functional ingredients for food and nutraceutical applications.

Acknowledgements: Gelpixe, Alimentos Congelados S.A. (Loures, Portugal), Vivid Foods, Lda (Atalaia, Portugal), and ArtesanalPesca—Organização de Produtores de Pesca, C.R.L. (Sesimbra, Portugal) for supplying the co-products.

Funding: This work was developed within the scope of the “BLUE BIOECONOMY INNOVATION PACT” (Project No. C644915664-00000026) funded by NextGenerationEU, under the incentive of “Agenda for Business Innovation” of the recovery and resilience plan (PRR). This work was also supported by Portuguese Foundation for Science and Technology (projects: UIDB/04077/2020, UIDB/ 04423/2020 and UIDP/04423/2020).

References:

- [1] Fish Matter Project webpage, URL: <https://fishmatter.pt/> (accessed 1.29.25)
- [2] M. Sapatinha et al., *Marine Drugs*, 23 (2025) 14.
- [3] C. Pires et al., *Foods*, 13 (2024) 2418.

Study of the phenolic composition of wines from grapevines irrigated with treated wastewater

Cátia Costa^{1,*}, Igor Gonçalves², Maria Manuel Maia³, Cristina Carlos¹, Ana Alexandra Oliveira¹ and Ana Novo Barros¹

¹ Centre for the Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Universidade Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

² Douro Viticulture Development Association (ADVID), 5000-033 Vila Real, Portugal

³ Poças – Sociedade Vinícola Terras de Valdigem (SVTV), 4400-337 Vila Nova de Gaia, Portugal

* catiac@utad.pt

Water scarcity in the agricultural sector is a current problem that requires effective solutions, so as not to compromise food production for the world population, which is expected to reach 9 billion inhabitants by 2050 [1,2]. In Portugal, the *Região Demarcada do Douro*, where viticulture predominates, has suffered from water scarcity [3], making it necessary to resort to irrigation of vineyards so that production does not decrease [4]. Additionally, several countries have chosen to irrigate vineyards with treated wastewater in order to mitigate the problem of water shortage. The objective of this work was outlined following the problem previously exposed, through a partnership between 4 companies and the University of Trás-os-Montes and Alto Douro, which developed a project to irrigate vineyards with treated urban wastewater (TWW), with different irrigation percentages (30% and 60% crop evapotranspiration (Etc)), in the Douro Superior sub-region. The wines produced from the resulting grapes were analyzed for their phenolic composition using HPLC–DAD–ESI–MS/MS to assess the impact of treated wastewater irrigation on phenolic profiles in comparison to wines derived from grapes irrigated with drinking water. After this analysis, it was observed that wines produced from grapes irrigated with drinking water (water hole 30% ETc and water hole 60% ETc) and with treated wastewater (TWW 30% ETc and TWW 60% ETc) presented a similar phenolic profile, however, both were different from that observed in wines obtained from grapes that were not irrigated.

Acknowledgments: This work was supported by “La Caixa” Foundation under the project (Protocolo Promove_PL23-00026), the ARQUIMÉDES Doctoral Scholarship and National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UID/04033 and LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>).

References:

- [1] Peng, X.; Jiang, Y.; Chen, Z.; Osman, A.I.; Farghali, M.; Rooney, D.W.; Yap, P.-S. *Environ Chem Lett* (2023), 21, 765–801
- [2] Yang, M.; Chen, L.; Wang, J.; Msigwa, G.; Osman, A.I.; Fawzy, S.; Rooney, D.W.; Yap, P.-S. *Environ Chem Lett* (2023), 21, 55–80
- [3] Prada, J.; Dinis, L.T.; Soriato, E.; Vandelle, E.; Soletkin, O.; Uysal, Ş.; Dihazi, A.; Santos, C.; Santos,
- [4] J.A. Mitig Adapt Strateg Glob Chang (2024), 29
- [5] Wu, J.; Drappier, J.; Hilbert, G.; Guillaumie, S.; Dai, Z.; Geny, L.; Delrot, S.; Darriet, P.; Thibon, C.; Pieri, P. *OENO One* (2019), 53.

Impact of non-thermal technologies on whey stabilization at pilot scale

Sara Dias¹, Viviana Monteiro¹, Diogo Gonçalves¹, Carlos A. Pinto², Jorge A. Saraiva² and Marco Alves¹ ₁

¹ INOV.LINEA/TAGUSVALLEY – Science and Technology Park, Abrantes, Portugal;
marco_alves@tagusvalley.pt

² LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;
carlospinto@ua.pt, jorgesaraiva@ua.pt

Whey, a byproduct of the dairy industry, has a considerable environmental impact due to its high organic load, which can deplete oxygen in aquatic ecosystems and disrupt ecological balance [1]. To mitigate this issue, circular economy principles have been applied to valorise whey in new life cycles through its reintegration in food production systems for new products' development. A crucial step in this process is microbiological stabilization, which ensures safety and extends usability and shelf-life [2]. The aim of this study was to analyse the impact of different processing technologies, at a pilot scale, in microbiological and physicochemical properties of liquid whey, namely ultrasound (US, 20 KHz, 600 kJ.L⁻¹, 50 °C), laminar flow ultraviolet radiation (UV, 337 mJ.cm⁻², 3254 J.L⁻¹, 3 mW.cm⁻², 35 °C), high pressure processing (HPP, 600 MPa, 5 min, 18 °C) and conventional pasteurization (CP, 70 °C, 25 min). Processed samples were compared to non-processed liquid whey (NPLW) through microbiological analyses (total aerobic mesophiles, psychrophiles, thermophiles and lactic acid bacteria) and physicochemical analyses (pH, viscosity, precipitate). All treatments significantly reduced the count of total aerobic mesophiles, with overall reductions of 4.05 (CP), 4.47 (UV), 1.97 (HPP) and 5.16 (US) log units relatively to NPLW. Regarding lactic acid bacteria, were observed logarithmic reductions of 4.31 (CP), 4.31 (HPP), 2.4 (US) relatively to NPLW. Psychrophiles and thermophiles final counts were 13 CFU/mL or undetectable levels. Physicochemical analyses revealed a reduction of the amount of precipitate by 37% with US, 22% with HPP, and 28% with CP, when compared with NPLW. No changes were observed in pH and viscosity. UV treatment had the biggest increase of all treatment with the amount of precipitate 114% higher than NPLW. In future studies, shelf-life assessment and structural analysis of proteins processed by different methods should be conducted.

Acknowledgments: The authors gratefully acknowledge Queijaria Santiago (Portugal) for providing the liquid fresh whey.

Funding: This work received support from PRR—Plano de Recuperação e Resiliência, by the NextGenerationEU funds at TAGUSVALLEY, through the scope of the Agenda for Business Innovation “Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (VIAFOOD)” (Project no. 37 AAC n.º 02/C05i01/2022 with the application C644929456-00000040) and FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds.

References:

- [1] K. Lappa, A. Papadaki, V. Kachrimanidou, A. Terpou, D. Koulouglitis, E. Eriotou & N.
- [2] Kopsahelis, Cheese Whey Processing: Integrated Biorefinery Concepts and Emerging Food Applications. *Foods*, 8(8), 347, (2019).
- [3] D. Rocha-Mendoza, E. Kosmerl, A. Krentz, L. Zhang, S. Badiger, G. Miyagusuku-Cruzado, A. Mayta-Apaza, M. Giusti, R. Jiménez-Flores & I. García-Cano, Invited review: Acid whey trends and health benefits. *J. Dairy Sci.*, 104(2), 1262–1275, (2021).

From winery waste to oral health: Grape stem polyphenols as a sustainable treatment for periodontal diseases

Sofia Mariana Saavedra^{1,2*}, Gustavo Fernandes³, Javier Flores⁴, Ana Novo Barros⁵

¹University of Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal

² PhD student - University of Salamanca, Department of Surgery, Spain

³A.T. Still University - Missouri School of Dentistry and Oral Health, MO, USA

⁴University of Salamanca, Department of Surgery, Spain

⁵Centre for Research and Technology of Agroenvironmental and Biological Sciences, CITAB, Inov4Agro, Universidade Trás-os-Montes e Alto Douro, UTAD, Quinta de Prados, 5000-801 Vila Real, Portugal

*marianasaavedrasilva86@gmail.com

The wine industry faces challenges in managing substantial quantities of byproducts, mainly grape stems. 1 They have demonstrated favorable application in dentistry. Thus, this study aimed to explore the innovative use of grape stem polyphenols as a sustainable approach to managing periodontal diseases better. The recent scientific literature reveals that grape stem extracts, rich in polyphenols, exhibit potent antibacterial and anti-biofilm activities against key periodontal pathogens, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum*. These polyphenolic compounds significantly inhibit bacterial adhesion, a crucial step in pathogen colonization. Moreover, these byproduct-derived polyphenols show remarkable anti-inflammatory properties, suppressing proinflammatory cytokines production; exhibit potent antioxidant activities, effectively scavenging reactive oxygen species and enhancing cellular antioxidant defenses, mitigating oxidative stress-induced damage in periodontal tissues; 2 and they can inhibit osteoclastogenesis while promoting osteogenic differentiation, potentially preserving alveolar bone integrity. Furthermore, their oral application could also favorably modulate the periodontal microbiome.^{1,2} Thereby, utilizing grape stems for oral health applications presents a promising solution to wine industry waste management and a sustainable way to treat periodontal diseases; in addition, this approach aligns with circular economy principles, transforming a low-value byproduct into a high-value resource for healthcare, reducing environmental impact and creating new economic opportunities. These findings suggest that grape stem polyphenols represent a sustainable, multi-targeted approach to periodontal disease management. Their natural origin, broad spectrum of beneficial activities, and contribution to waste valorization make them attractive candidates for developing novel, eco-friendly therapeutic strategies. 3,4 Future research should focus on optimizing extraction methods, assessing environmental benefits, and conducting clinical trials to harness the potential of grape wholly stem polyphenols in periodontal health and sustainable resource management.

References

- [1] Leal, C., Santos, R. A., Pinto, R., Queiroz, M., Rodrigues, M., Saavedra, M. J., Barros, A., & Gouvinhas, I. (2020). Recovery of bioactive compounds from white grape (*Vitis vinifera* L.) stems as potential antimicrobial agents for human health. *Saudi Journal of Biological Sciences*, 27(4), 1009–1015.
- [2] Sánchez, M. C., Ribeiro-Vidal, H., Esteban-Fernández, A., Bartolomé, B., Figuero, E., Moreno-Arribas, M. V., Sanz, M., & Herrera, D. (2019). Antimicrobial activity of red wine and oenological extracts against periodontal pathogens in a validated oral biofilm model. *BMC Complementary and Alternative Medicine*, 19, 145
- [3] Gouvinhas, I., Santos, R. A., Queiroz, M., Leal, C., Saavedra, M. J., Domínguez-Perles, R., Rodrigues, M., & Barros, A. I. R. N. A. (2018). Monitoring the antioxidant and antimicrobial power of grape (*Vitis vinifera* L.) stems phenolics over long-term storage. *Industrial Crops & Products*, 126, 83–91
- [4] Ruiz-Moreno, M. J., Raposo, R., Cayuela, J. M., Zafrilla, P., Piñeiro, Z., Moreno-Rojas, J. M., Mulero, J., Puertas, B., Giron, F., Guerrero, R. F., & Cantos-Villar, E. (2015). Valorization of grape stems. *Industrial Crops and Products*, 63, 152–157.

Stabilization of watermelon albedo and evaluation of its potential as a dehydrated snack

Ana C.O. Soares¹, Maria I. Nora¹, Sara Ferreira¹, Francisco A. Da Silva F.², Sónia Ferreira³, Sílvia Petronilho⁴, Manuel A. Coimbra³, Cláudia Passos³

¹ Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

² CICECO and ³ LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

⁴ LAQV-REQUIMTE & CICECO, Department of Materials and Ceramic Engineering, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

* cpassos@ua.pt

Watermelon albedo, the white part of the rind, is often discarded as waste, but it holds significant potential for various food applications due to its rich biomolecules content and their bioactive potential. Considering these issues, this work aims to use albedo, after stabilization by dehydration, to develop a snack rich in pectic polysaccharides and citrulline.

A Microwave Hydrodiffusion and Gravity (MHG) equipment was used to stabilize the samples by dehydrating the initial watermelon albedo [1] by applying different power conditions. Simultaneously, the material diffused in the extracts was collected and their content in free sugars and citrulline was evaluated. The dehydrated albedo was also ground into flour and subsequently characterized for its content of polymeric and free sugars, uronic acids, and methyl esterification degree of pectic polysaccharides. For sensory analysis, the dehydrated albedo was treated as a “snack”. A non-trained panel used a hedonic scale to measure the sensory quality of the snack using 5 parameters: 1—dislike a lot, 2—dislike, 3—indifferent, 4—like, and 5—like a lot. The evaluation form given to the panellists listed different parameters with scores ranging from 1 to 5. The dehydration process allowed the recovery of extracts containing citrulline and free sugars in their composition, although most of them remained in the dehydrated samples. The powder chemical composition revealed polymeric sugars correlated with pectin, associated with the presence of uronic acids, potentially with regions of xylogalacturonans and rhamnogalacturonans, due to the presence of xylose, galactose and rhamnose residues. This identified albedo as a potential source of pectic polysaccharides. The sensorial results showed a positive position towards the snack, with space for improvement (e.g. using sweet/salt taste-enhancers). In conclusion, this study identified an opportunity for watermelon albedo use, creating a food product with potential nutritional value when stabilized through the dehydration process.

Acknowledgments: Thanks are due to University of Aveiro and to the projects of LAQV/REQUIMTE (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020) and CICECO-Aveiro Institute of Materials, UIDB/50011/2020 (DOI 10.54499/UIDB/50011/2020), UIDP/50011/2020 (DOI 10.54499/UIDP/50011/2020) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020), funded by FCT/MCTES through national funds. FCT is also thanked for the individual Call to Scientific Employment Stimulus (CPP, <https://doi.org/10.54499/CEECIND/00813/2017/CP1459/CT0053>). S.S.F. thanks its contract to the project VIIAFOOD - Plataforma de Valorização, Industrialização e Inovação comercial para o AgroAlimentar (n.º C644929456-00000040). SP thanks its contract to the project “FAIST – Fábrica Ágil, Inteligente, Sustentável e Tecnológica” (Project no.66 with application C644917018-00000031).

References:

- [1] P.A.R. Fernandes, R. Bastos, J. Calvão, F. Neto, E. Coelho, D.F. Wessel, S.M. Cardoso, M.A. Coimbra, C.P. Passos. *Bioresource Technology* 333 (2021), 125207. <https://doi.org/10.1016/j.biortech.2021.125207>

Active films incorporating α -tocopherol in polymeric matrix by extrusion and ultrasonic spray coating

**Ana Gabriela Azevedo^{1*}, Victor Souza¹, Ana Isabel Bourbon¹, Graça Brotas²,
Tiago Filipe², Pablo Fucinos¹, Lorenzo Pastrana¹ and Miguel Cerqueira¹**

¹ INL - International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n 4715-330, Braga, Portugal

² Silvex - Indústria de Plásticos e Papéis, S.A., Rua das Camélias nº 7, 2130-233, Benavente, Portugal

* ana.azevedo@inl.int

Active packaging can be used to increase the food products' shelf-life, while maintaining their nutritional quality, ensuring safety and preventing food waste. Active packaging is produced with an active agent that interacts with the food, being the active agent added to the packaging by two main strategies: the incorporation into the polymeric matrix by extrusion, or application on the film surface by coating technologies [1]. In this work, active films were produced by adding an antioxidant through two processes: extrusion and ultrasonic spray coating. The extrusion process is the most used process to produce food packaging, where the materials experience progressive heating and pressure until melting, and after the material cools, they obtain their shape. The ultrasonic spray coating process is a new technology that allows the production of thin film layers on substrates at room temperature. The materials used to produce films were polyethylene (PE) and bioplastic (Mater-bi®). The α -tocopherol (α -TOC) a vitamin with antioxidant properties, was selected as the active agent. After the production of the active films, the antioxidant activity, mechanical and optical properties were studied. Moreover, the dispersion of coatings was evaluated by scanning electronic microscopy (SEM). Results showed that it is possible to incorporate α -TOC into polymeric films using the extrusion process and the ultrasonic spray coating. The SEM images showed a good distribution of the active agent by both processes. The active agent showed a good antioxidant activity when to the films by extrusion and ultrasonic spray. However, the ultrasonic spray coating strategy, where temperature is not used, showed to be the most promising in maintaining the antioxidant properties of the TOC.

Acknowledgments: The authors acknowledge the financial support of the project “Agenda VIIAFOOD: Platform for the Agri-Food Valorisation, industrialization and commercial innovation”, with the reference n.ºCC644929456-00000040, co-funded by Component C5 – Capitalisation and Business Innovation under the Portuguese Resilience and Recovery Plan, through the NextGeneration EU Fund. . Ana Isabel Bourbon acknowledges funding by FCT, through the individual scientific employment program contract (2020.03447.CEECIND).

References:

- [1] Azevedo, A.G.; Barros, C.; Miranda, S.; Machado, A.V.; Castro, O.; Silva, B.; Saraiva, M.; Silva, A.S.; Pastrana, L.; Carneiro, O.S.; et al. Active Flexible Films for Food Packaging: A Review. *Polymers* 2022, 14, 2442

Characterisation of apple and ginger pomace for potential syrup production: Nutritional composition, antioxidant capacity, and reducing sugar profile

Joana Rodrigues Gonçalves^{1,*}, Ana Maria Gomes¹, Maria Manuela Pintado¹

¹Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

* jrgoncalves@ucp.pt

Fruit and vegetable by-products account for 44% of global food waste yet hold potential for food innovation. Apple pomace, a by-product of apple juice extraction, consists of flesh and skin (94.5%), seeds (4.1%), and stems (1.1%). Ginger residue, a by-product of the herbal medicine and beverage industries, is rich in dietary fiber and bioactive compounds with anti-inflammatory and anti-cancer properties. Apple and ginger pomace, generated during juice extraction and food processing, contains significant amounts of bioactive compounds, minerals, dietary fiber, and carbohydrates, offering potential applications in sustainable food ingredient development.

This study characterises an apple and ginger pomace from the production of juice, from Frubaça (Copa, Alcobaça, Portugal), to evaluate its potential for producing a sugar-rich syrup with functional properties. Nutritional composition was assessed using AOAC methods, while total phenolic compounds were determined using the Folin-Ciocalteu method. Associated antioxidant activity was determined via ABTS and DPPH assays. The reducing sugars were quantified by High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD). Apple and ginger pomace contains a significant sugar content, with glucose and fructose representing 22.2 ± 2.36 g/100 g and 9.71 ± 1.42 g/100 g DM. Additionally, the presence of phenolic compounds (5.31 ± 0.54 mg GAE/g DM, free phenolics) suggested potential health benefits, corroborated by its antioxidant potential (ABTS 7.98 ± 1.69 mg TE/g DM and DPPH 5.33 ± 0.89 mg TE/g DM), making this by-product suitable for functional food applications and supporting its valorisation in syrup production. Developing a sugar-rich syrup from apple and ginger pomace could provide an alternative to conventional sweeteners while incorporating antioxidant properties. Future research will focus on optimizing the extraction of reducing sugars. This study highlights the potential of apple and ginger pomace as a sustainable ingredient, contributing to waste reduction and the development of innovative food products with added nutritional, functional value with sustainable character.

Acknowledgments: This work was supported by the National Funds from FCT - Fundação para a Ciência e Tecnologia by FCT individual PhD grant (2022.12167.BD) and TRANSCOLAB (0612_TRANS_CO_LAB_2_P). The authors thank to FCT and CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado.

References:

- [1] Z. Ali, et al., Chem Sci Rev Lett, 12 (2023) 49.
- [2] H.N. Rabetafika, B. Bchir, C. Blecker, A. Richel, Trends Food Sci Technol, 40 (2014) 99.
- [3] V.I. Candrawinata, J.B. Golding, P.D. Roach, C.E. Stathopoulos, CYTA - J Food, 13 (2015) 293
- [4] B.Z.R. Araujo, V.F.R. Martins, M.E. Pintado, R.M.S.C. Morais, A.M.M.B. Morais, Processes, 12 (2024) 1096

Consumer perception of a new sustainable food product made from discarded table grapes

Mariana Fonseca¹, Pedro Sousa^{1,2}, Luís Miguel Cunha^{1,2}, Susana Caldas Fonseca^{1,2,*}

¹ DGAOT, Faculty of Sciences, University of Porto, Porto, Portugal

² GreenUPorto/Inov4Agro, Faculty of Sciences, University of Porto, Vila do Conde, Portugal

*susana.fonseca@fc.up.pt

Around 15% of fruits and vegetables are estimated to be discarded and wasted in Europe, representing a significant environmental and economic problem. In Portugal, the production of table grapes contributes to this, with fresh fruit companies recording losses of up to 25% due to rejection for quality reasons. This study aimed to evaluate the consumer perception of implementing a circular economy approach to developing new food products, particularly developing a new product incorporating industry-discarded table grapes from Portugal.

An online questionnaire was carried out among Portuguese consumers, including a free word association task related to the concepts: 'Sustainable Food' and 'Upcycled Food', attitudes towards fruit leather rolls and grape leather rolls, and determinants of acceptance and consumption behaviour.

The study revealed a positive perception among consumers regarding consuming upcycled foods despite the lack of knowledge inherent in this type of product. There was also a positive correlation between consumers with sustainable attitudes and their intention to consume fruit leather rolls made from discarded table grapes (Spearman's coefficient = 0.257; $p < 0.001$).

This study contributes to the idea that there are good prospects for the Portuguese market to accept new food products based on table grapes that would otherwise go to waste, thus reducing the sector's environmental impact and promoting the circular economy.

Acknowledgements: Authors acknowledge support from the strategic project UID 05748: GreenUPorto - *Centro de investigação em Produção Agroalimentar Sustentável*, funded by national funds via FCT (Foundation for Science and Technology).



Valorisation of grape stems: Polyphenolic profile and inhibition of skin-aging enzymes for cosmetic and pharmaceutical applications

Rui Dias-Costa^{1,*}, Marta Coelho², Concepción Medrano-Padial³, Raquel Fernandes¹, Raúl Domínguez-Perles³, Irene Gouvinhas¹, Ana Novo Barros¹

¹ Centre for the Research and Technology of Agro-Environmental and Biological Sciences, CITAB, Inov4Agro, University of Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal

² Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal

³ Laboratorio de Fitoquímica y Alimentos Saludables (LabFAS), Centro de Edafología y Biología Aplicada del Segura-Spanish Council for Scientific Research (EBAS-CSIC), University Campus of Espinardo 25, 30100 Murcia, Spain.

* ruiacosta@utad.pt

In Portugal, a country with a rich winemaking tradition, the wine production chain generates a considerable amount of winery by-products (WBPs), including grape stems, which are produced in large quantities over a short period. During the destemming phase, approximately 30 kg of stems are produced for every 1000 kg of grapes harvested [1]. This presents a promising opportunity for valorization, aimed at developing innovative products for the industrial sector, with potential health benefits due to their rich polyphenolic composition.

In this work, the polyphenolic profile of extracts from grape stems of seven white grape varieties collected from the Região Demarcada do Douro was determined using high-performance liquid chromatography coupled with a photodiode array detector and electrospray ionization multi-stage mass spectrometry (HPLC-PDA-ESI-MSn). The ferric-reducing antioxidant power (FRAP) and radical scavenging capacity were also assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radicals. Furthermore, the study investigated the anti-aging and skin depigmenting activities of these extracts. In this study, proanthocyanidins and catechin derivatives (PCDs), phenolic acids, and flavonols were identified and quantified. Among the varieties studied, the extracts from 'Códaga do Lariño' presented one of the highest polyphenol concentrations. It also showed the highest results for antioxidant capacity and elastase and tyrosinase inhibition. Pearson's correlation analysis showed a significant positive correlation between certain PCDs with both FRAP and DPPH assays, as well as between the identified flavonols and anti-elastase activity [2]. These findings highlight the potential health benefits of grape stem polyphenols to promote antioxidant and anti-aging activity, thereby substantiating their usage across several industries.

Funding: This work is supported by National Funds by FCT –Portuguese Foundation for Science and Technology, under the projects UID/04033 and LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>).

Acknowledgments: Rui Dias-Costa acknowledges the financial support provided by National Funds by Fundação para a Ciência e Tecnologia (FCT) Ph.D. grant: 2021.07571.BD. Irene Gouvinhas thanks FCT for funding through the Scientific Employment Stimulus—Individual Call (2022.00498.CEECIND), <https://doi.org/10.54499/2022.00498.CEECIND/CP1749/CT0001> (accessed on 17 May 2024).

References:

- [1] R.P. Rodrigues, L. M. Gando-Ferreira, M. J. Quina, *Molecules* (2022), 27.
- [2] R. Dias-costa, C. Medrano-padial, R. Fernandes, R. Domínguez-Perles, I. Gouvinhas, A. N. Barros, *Molecules* (2024), 1–22.

Impact of previous exposure to fraud on Portuguese consumer perceptions and attitudes towards food fraud

Isabel Sousa^{1,2,3†}, Maria João Costa^{1,2†}, Ana Pinto de Moura^{1,4}, José António Teixeira³ and Luís Miguel Cunha^{1,2*}

¹GreenUPorto- Sustainable Agrifood Production Research Centre/INOV4Agro, University of Porto, Vila do Conde, Portugal

²DGAOT, Faculty of Sciences, University of Porto, Porto, Portugal

³CEB-Centre of Biological Engineering, University of Minho, Campus Gualtar, 4710-057, Braga, Portugal

⁴DCeT, Universidade Aberta, Porto Portugal

[†]Equal contribution

*lmcunha@fc.up.pt

Consumers are becoming increasingly sceptical and cautious about the foods they buy and consume [1,2]. The attitudes and behaviours of Portuguese consumers about food chain fraud remain little understood [3,4]. This study evaluates consumers' perceptions, attitudes, and reported behaviours toward food fraud. A total of 368 participants, recruited through digital devices, fully answered a self-reported questionnaire [5] in LimeSurvey. Descriptive statistics, χ^2 test, the Mann-Whitney test, or the Wilcoxon test were used, where appropriate. Factor analysis was applied to reduce the main dimensions of the questionnaire, and binary logistics regression was also computed to evaluate the determinants of carrying out self-protective behaviours to minimise the risk of exposure to food fraud.

Consumers are generally aware of the motivations of food fraud perpetrators, namely the motivation to obtain greater profits. They also reveal great concerns about health in the context of food fraud, emphasising measures to prevent it; they also present low levels of trust in products, systems, and authorities. This is emphasised by consumers previously exposed to food fraud. Meat, fish, poultry, and dietary supplements represent the categories most vulnerable to food fraud, and e-commerce, traditional markets, and foodservice channels are the most susceptible stages in the food supply chain. Older consumers and women appear to understand food fraud better. Although consumers intend to report food fraud when facing a food fraud situation, only a few have been reported by official channels. Self-protective behaviours to minimise the risk of exposure to food fraud include the type of store, brand, packaging, product origin, and search for food fraud information. Consumer's perception of fraudsters' motivations, health concerns, measures to prevent food fraud, level of trust in products, systems and authorities, age, and previous exposure to fraud seem to be involved in adopting these behaviours.

Acknowledgments: Acknowledgments: Authors Costa, Moura, and Cunha acknowledge support from the strategic project UID 05748: GreenUPorto - Centro de investigação em Produção Agroalimentar Sustentável, funded by national funds via FCT (Foundation for Science and Technology - Portuguese Ministry of Science, Technology and Higher Education).

References:

- [1] H. Kendall, B. Clark, C. Rhymer, S. Kuznesof, J. Hajslova, M. Tomaniova, P. Brereton, L. Frewer, L. Trends in Food Science and Technology, 94 (2019)
- [2] J. Barnett, F. Begen, S. Howes, A. Regan, A. McConnon, A. Marcu, S. Rowntree, W. Verbeke, Food Control, 59 (2016)
- [3] M.J. Costa, I. Sousa, A. Pinto Moura, J.A. Teixeira, L.M. Cunha, Journal of Food Protection, 87(2024)7.
- [4] M.J. Moreira, J. García-Díez, J.M.M.M. de Almeida, C. Saraiva, Foods, 10 (2021) 5
- [5] Sousa, I.F.S.F., Fraude alimentar em Portugal: Perceção e Comportamento dos Consumidores [Dissertação de Mestrado]. Braga, Universidade do Minho e Faculdade de Ciências da Universidade do Porto (2022) 230 p.

Control of *Stemphylium vesicarium* in “Rocha” Pear Using Encapsulated Essential Oils

Inês Mendonça^{1,2,5}, Beatriz Fernandes^{3,4}, Carina Almeida^{1,7,8}, Carmo Serrano^{3,9,10}, Miguel Leão², Armando Venâncio^{5,6}, Sónia Carina Silva^{5,6,*}

1 INIAV, IP – National Institute for Agrarian and Veterinary Research, Polo de Vairão, Vila do Conde, Portugal

2 INIAV, IP – National Institute for Agrarian and Veterinary Research, Polo de Alcobaca, Alcobaca, Portugal

3 INIAV, IP – National Institute for Agrarian and Veterinary Research, Oeiras, Portugal

4 CERENA, DEQ, Instituto Superior Técnico, University of Lisbon, Lisbon, Portugal

5 CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal

6 LABBELS – Associate Laboratory, Braga/Guimarães, Portugal

7 LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

8 AliCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Porto, Portugal

9 LEAF – Linking Landscape, Environment, Agriculture and Food Research Unit, Associate Laboratory

10 TERRA, Instituto Superior de Agronomia, University of Lisbon, Lisbon, Portugal

* soniasilva@ceb.uminho.pt

Stemphylium vesicarium is a plant-pathogenic fungus that poses a significant challenge to pear production, where it causes brown spot of pear (BSP) [1]. The “Rocha” pear, an exclusively Portuguese variety of high importance, has been experiencing substantial losses due to BSP [2]. The rise of fungicide resistance and concerns about their environmental impact have driven the search for sustainable control strategies. In this context, essential oils (EOs) have emerged as eco-friendly alternatives to synthetic fungicides due to their strong fungicidal properties [3]. This study aimed to evaluate the efficacy of three essential oils - lemon eucalyptus, prince herb and lemon thyme - in inhibiting the growth of *S. vesicarium* isolated from “Rocha” pear showing BSP symptoms. To mitigate the volatility and instability of these compounds, the oils were encapsulated with HI-CAP® 100, a modified starch-based encapsulating agent. In vitro, atomized samples of these three encapsulated EOs – each at concentrations of 15% and 30% – were tested against *S. vesicarium* strains. The antifungal activity of these encapsulated EOs was assessed by culturing *S. vesicarium* on potato dextrose agar (PDA) supplemented with varying concentrations of the formulations: 80 mg/mL, 40 mg/mL, 20 mg/mL, 10 mg/mL and 1 mg/mL. At 15%, prince herb and lemon thyme completely inhibited fungal growth at a minimum concentration of 20 mg/mL, while at 30%, they were effective at a minimum concentration of 10 mg/mL. In contrast, lemon eucalyptus failed to achieve complete inhibition of strain growth at any concentration tested. Sustainable strategies that reduce reliance on synthetic products are crucial for ensuring food security while safeguarding both the environment and human health [3]. This study demonstrated that encapsulated essential oil effectively control the growth of *S. vesicarium* from “Rocha” pear at low concentrations, highlighting their potential as a natural alternative to synthetic fungicides.

Acknowledgments: This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/2020 unit, with DOI 10.54499/UIDB/04469/2020, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020. Inês Mendonça also acknowledges FCT for the fellowship 2023.04778.BDANA. The authors would also like to acknowledge FCT for supporting the project “Pacto da Bioeconomia Azul” (PRR-C644915664-00000026).

References:

- [1] F. Cavina, S. Baiocco, L. Tomba, F. Ravaglia, C. Moretti, R. Raso, V. G. Rettore, M. Parrili, G. Pradoles, *R. Bugiani, Agronomy*, 14 (2024) 11.
- [2] M. Loebler, C. Sánchez, E. M. Maurício, E. Diogo, M. Santos, P. Vasilenko, A. S. Cruz, B. Mendes, M. Gonçalves, M. P. Duarte, *Applied Sciences (Switzerland)*, 10 (2020) 6.
- [4] T. Szczygieł, A. Koziróg, A. Otlewska, *Molecules*, 29 (2024) 16.

From Waste to Value: Antioxidant and Antimicrobial Properties of Fresh and Dried Cardoon Leaf Extracts

Cássia H. Barbosa^{1,2}, Mariana A. Andrade^{1,3}, Maria Paula Duarte², Ana Rita Mateus^{4,5}, Sidney Tomé¹, Fernanda Vilarinho¹, Ana Luísa Fernando², Ana Sanches Silva^{4,6,7}

¹ National Institute of Health Doutor Ricardo Jorge, Department of Food and Nutrition, Av. Padre Cruz, 1649-016 Lisbon, Portugal

² METRICs, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

³ REQUIMTE/LAQV, Rua D. Manuel II, Apartado 55142, Porto, Portugal

⁴ University of Coimbra, Faculty of Pharmacy, Coimbra, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal

⁵ National Institute of Agrarian and Veterinary Research (INIAV), I.P., Rua dos Lagidos, Lugar da Madalena, Vairão, Vila do Conde 4485-655, Portugal

⁶ Centre for Study in Animal Science (CECA), ICETA, University of Porto, Porto, Portugal

⁷ Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal

* cassia.barbosa@insa.min-saude.pt

Cynara cardunculus L. (cardoon) is a resilient perennial crop that is highly productive under extremely challenging conditions. Even though its flowers are used in the cheese-making process, its bioactive- rich leaves are largely discarded ^[1]. This study assessed the antioxidant and antimicrobial potentials of fresh and dried cultivated cardoon leaves (var. *altalis* DC) using ethanolic and methanolic extracts as well as the effects of oven-drying with forced air circulation on the bioactive profile. Dried leaf extracts showed better antioxidant activity than fresh leaf extracts, despite having a greater polyphenol profile. A total of 16 compounds were identified, and luteolin, apigenin, and chlorogenic acid were the most prevalent. The highest antioxidant activity was presented by dried leaf extracts for both the methanolic (EC₅₀ = 0.8 mg/mL, AAC = 279.67) and ethanolic (EC₅₀ = 2.1 mg/mL, AAC = 448.06) solvents.

Gram-positive bacteria were more sensitive to ethanolic and methanolic extracts than Gram-negative bacteria. With lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (125–2000 µg/mL) across most of tested bacteria, the dried leaf ethanolic extract showed greater antimicrobial activity. Although *Aspergillus fumigatus* was only mildly inhibited, dried leaf extracts demonstrated superior antimicrobial effectiveness compared to fresh leaf extracts.

These studies demonstrate the potential benefits of using dried cardoon leaves in the food, cosmetic, and pharmaceutical industries because of the bioactive components they have. These findings encourage sustainable production and consumption, which align with the goals of the United National Sustainable Development Goal 12, as well as the principles of circular economy. Cardoon leaves can be further transformed into relevant products in the current industrial world while simultaneously ensuring economic and environmental sustainability.

Acknowledgments: Cássia H. Barbosa thanks the Fundação para a Ciência e Tecnologia (FCT), Portugal, for the Ph.D. Grant 2021.08154.BD. The authors would like to thank the company NINA, Lda, for kindly supplying the cardoon leaves. **Funding:** This work was supported by the Mechanical Engineering and Resource Sustainability Center—METRICs, financed by national funds from the FCT/MCTES (<https://doi.org/10.54499/UIDB/04077/2020>, <https://doi.org/10.54499/UIDP/04077/2020>). This research was funded by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the grant UIDB/00211/2020 and 2023.15813.PEX.

References:

- [1] C.H. Barbosa, M.A. Andrade, F. Vilarinho, I. Castanheira, A.L. Fernando, M.R. Loizzo, A. S. Silva, *Foods*, 9 (2020) 564.

Turning agri-food by-products into functional ingredients: sustainable innovations for waste reduction

**Ana Paupério¹, María Emilia Brassesco¹, Carlos D. Pereira², João Paulo Ferreira¹,
Manuela Pintado^{1*}**

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina –
Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005,
Porto, Portugal

² Escola Superior Agrária de Coimbra, Bencanta, 3045-601, Coimbra, Portugal

* mpintado@ucp.pt

Background: The food industry generates significant by-products, contributing to environmental challenges and resource inefficiencies. In response, there is growing interest in valorising these by-products, recognising their potential as sources of valuable compounds with economic and nutritional benefits ⁽¹⁾. Among these, brewer's spent yeast (BSY) and okara are promising residues rich in proteins, fibres, and other bioactive compounds ^(2, 3). These by-products align with sustainability and circular economy principles, making them ideal candidates for transformation into high-value food ingredients.

Methods: This study focused on valorising BSY and okara by-products through integrated processing to obtain alternative protein-rich ingredients. Pre-treatment methods such as drying, milling, and thermal hydrolysis were employed to optimise the extraction of nutrients and bioactive compounds to enhance the functional properties of the resulting ingredients. The extracted ingredients were comprehensively characterised for their protein content, fibre composition, antioxidant activity, and techno-functional properties. Subsequently, salty cracker prototypes were developed by incorporating these functional ingredients. The physicochemical properties of the crackers were evaluated, including texture (assessed via texture profile analysis), colour (measured using the CIELAB system), and water activity. These analyses aimed to determine the suitability of the ingredients for food applications and their impact on the final product quality.

Results: The developed functional ingredients exhibited high nutritional value, particularly in protein and dietary fibre content. Crackers formulated with these upcycled ingredients demonstrated desirable textural properties, supporting their potential as sustainable, health-promoting snacks.

Conclusion: This research underscores the feasibility of using food industry by-products as valuable functional ingredients for innovative food applications. By integrating BSY and okara into cracker formulations, this study promotes resource efficiency, contributes to sustainability goals, and aligns with the principles of the circular economy in food production.

Keywords: Food by-products, functional ingredients, sustainable food innovation.

Acknowledgments: This work was funded by Agenda VIIAFOOD - Platform for Valorisation, Industrialization and Commercial Innovation for Agro-Food (no. C644929456-00000040)

References:

- [1] Gómez-García R, Campos DA, Aguilar CN, Madureira AR, Pintado M. Valorisation of food agro-industrial by-products: From the past to the present and perspectives. *Journal of Environmental Management*. 2021;299.
- [2] Jaeger A, Arendt EK, Zannini E, Sahin AW. Brewer's Spent Yeast (BSY), an Underutilized Brewing By-Product. *Fermentation* [Internet]. 2020; 6(4).
- [3] Asghar A, Afzaal M, Saeed F, Ahmed A, Ateeq H, Shah YA, et al. Valorization and food applications of okara (soybean residue): A concurrent review. *Food Science & Nutrition*. 2023;11(7):3631-40.

Incorporating chestnut shell fibers into biodegradable films for enhanced performance

Ana Pinto¹, Samara Rodrigues¹, Jorge Moreira², Valentina F. Domingues^{1*}, Elsa F. Vieira^{1*}

¹ REQUIMTE/LAQV, Polytechnic of Porto – School of Engineering, Porto, Portugal

² Sortegel - Produtos Congelados, S.A., Bragança, Portugal

*emfva@isep.ipp.pt, vfd@isep.ipp.pt

This study aimed to explore the potential of using chestnut shell fibers as a reinforcement agent in biodegradable starch-based film. Two different extraction methods for obtaining fibers from chestnut shells were compared and characterized: (i) the alkaline method, which has been used in previous studies [1] and involves a high concentration of 5% NaOH, producing residues that require further treatment, and (ii) the mechanical method, which is considered more sustainable and avoids the need for chemicals. The fibers obtained through both methods were analysed using FTIR-ATR, and since they showed similar characteristics, only the mechanically treated fibres were used to prepare films with 10% and 15% fiber content (by weight relative to starch). The commercial potato starch-based films, reinforced with chestnut shell fibers, were created using the Solvent Casting technique.

To simulate real-life conditions for food packaging, the films were exposed to four different combinations of temperature and light, reflecting the conditions in which the films might be used. They were evaluated for mechanical, optical, moisture, and solubility properties at four-time intervals: control (0 days), 2 days, 1 week, and 2 weeks.

The results demonstrated that the mechanical extraction method was a more sustainable option, as it eliminated the need for NaOH and reduced the generation of toxic residues. Overall, the exposure conditions did not significantly affect the films' mechanical, optical, or physicochemical properties compared to the control films.

Funding: This work received financial support from FCT/MCTES (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds and by the project Chestfilm, supported by “La Caixa” Foundation and Fundação para a Ciência e Tecnologia through the 4th edition of Promove program “O Futuro do Interior”. This work also received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

Acknowledgments: The authors are grateful to Sortegel for supplying chestnut shell material. Elsa F. Vieira acknowledge the FCT for her work contract (DOI 10.54499/CEECIND/03988/2018/CP1545/CT0006), supported by national funds.

References:

- [1] Amaral, L., Rodrigues, F., Silva, A., Costa, P., Delerue-Matos, C., & Vieira, E.F. Food Chemistry, 396 (2022) 133609.

Brewing Spent Yeast as a Sustainable Solution for Tannin Adsorption

Elsa F. Vieira^{1*}, Tomás Amaral², Jorge Moreira³, Tiago Brandão⁴, Cristina Delerue-Matos²

¹ REQUIMTE/LAQV, Polytechnic of Porto – School of Engineering, Porto, Portugal

² Polytechnic of Porto – School of Health, Porto, Portugal

³ Sortegel - Produtos Congelados, S.A., Bragança, Portugal

⁴ Super Bock Group, Via Norte, 4465-764 Leça do Balio, Portugal

**emfva@isep.ipp.pt, elsavieiraf@gmail.com*

This study explored the potential of brewing spent yeast (BSY) as an adsorbent for tannins from a chestnut shell extract (CS-tannin extract). The extract was obtained through an alkaline treatment (5% NaOH (v/v)) to recover cellulosic material from chestnut shells, which requires further valorisation. Various BSY treatments, including lyophilization, immobilization in calcium alginate beads, and alkaline and acid treatments, were tested to determine the best tannin adsorption capacity. The BSY material underwent characterization before and after the experiments, including point of zero charge (pHPZC) determination, Fourier transform infrared (FT-IR) analysis, and scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS).

Equilibrium was achieved within 10 minutes, with the highest biosorption capacity of CS-tannin extract observed in lyophilized BSY, which showed a value of 31.81 ± 3.08 mg Tannic Acid Equivalents g^{-1} yeast. The Sips isotherm model fitted well to the data, suggesting that tannin biosorption onto residual yeast cells is a chemisorption process. FT-IR analysis revealed several functional groups in the BSY, particularly carboxyl, amino/hydroxyl, and amide groups, which play a key role in tannin biosorption.

These results demonstrate that BSY, a valuable by-product of the brewing industry, is an effective biosorbent for tannins from the disposal solution resulting from chestnut shell cellulosic material extraction. Further research is needed to better understand the specific interaction mechanisms and explore the practical applications of tannin-enriched BSY.

Funding: This work received financial support from FCT/MCTES (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds and by the project Chestfilm, supported by “La Caixa” Foundation and Fundação para a Ciência e Tecnologia through the 4th edition of Promove program “O Futuro do Interior”. This work also received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

Acknowledgments: The authors are grateful to Sortegel for supplying chestnut shell material and to Super Bock Group for supplying spent yeast. Elsa F. Vieira acknowledges the FCT for her work contract (DOI 10.54499/CEECIND/03988/2018/CP1545/CT0006), supported by national funds.

References:

- [1] Amaral, L., Rodrigues, F., Silva, A., Costa, P., Delerue-Matos, C., & Vieira, E.F. Food Chemistry, 396 (2022) 133609.

[S&EC 49]

Upcycling spent coffee grounds: Green synthesis of nZVI for soil and water remediation

**Filipe Fernandes^{1,2}, Paula Paíga¹, Sónia Figueiredo¹, Cristina Delerue-Matos¹,
Clara Grosso^{1,*}**

¹REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto,
Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

²Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto,
Portugal

* *claragrosso@graq.isep.ipp.pt*

The agrifood industries are responsible for large amounts of waste generated every year. Spent coffee grounds (SCGs) have an estimated annual production of 60 million tons. The need for ways to enhance their valorization and reuse is immense. Aligned with the principles of circular economy, SCGs were used to produce antioxidant-rich extracts for the remediation of contaminated soils. Zero valent iron nanoparticles (nZVI) have been extensively researched for remediation, as these particles are highly reactive, and have been reported to have great remediation potential. In this work, the extracts of SCGs were used for the green synthesis of nZVI, with particle sizes below 100 nm. These nZVI were utilized for the remediation of soil and water contaminated with venlafaxine, an antidepressant increasingly more present in waters and soils. Different amounts of nZVI were utilized, at different concentrations of venlafaxine. The utilization of nZVI resulted in the removal of venlafaxine, even at a very high concentration (10 mg/L). The obtained results show the potential for the use of green synthesized nZVI from agrifood wastes in the removal of contaminants from waters and soils.

Acknowledgments: This work received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. Filipe Fernandes thanks FCT for the financial support through a PhD fellowship (2021.06806.BD, DOI 10.54499/2021.06806.BD) and Clara Grosso is thankful for her contract (2020.03436.CEECIND/CP1596/CT0008, DOI 10.54499/2020.03436.CEECIND/CP1596/CT0008) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.



From waste to value: Environmental impact assessment of chestnut-based candle holders

Simão Silva¹, Olga M. Freitas¹, Elena Surra², Ana R. Carreiras³, Púria Esfandiari³, João Francisco Silva³, Cristina Delerue-Matos¹, Valentina F. Domingues^{1,*}

¹REQUIMTE/LAQV, Porto, Portugal

²HyLab, Sines, Portugal

³ M4S, School of Engineering, Polytechnic of Porto, Porto, Portugal

* vfd@isep.ipp.pt

The excessive use of fossil-based plastics has led to severe environmental issues, including pollution and resource depletion [1]. To address this challenge, biocomposites derived from agro-industrial residues emerge as a promising alternative [2]. This study focuses on developing sustainable candle holders using non-commercial chestnuts, a significant waste product from the chestnut industry in Portugal. The biocomposites were produced via hot compression molding at 120 °C, with optimized formulations containing up to 75% chestnut and no glycerol, while a natural shellac-based bioresin was applied as a hydrophobic coating.

A comparative Life Cycle Assessment (LCA) was conducted to evaluate the environmental performance of different candle holder production scenarios. Scenario A represents the direct combustion of chestnuts for energy generation. Scenarios B1–B3 correspond to chestnut-based candle holders, with B1 including glycerol and shellac considering final landfilling, B2 excluding both additives but also ending in landfill, and B3 following the same formulation as B2 but incorporating recycling at the end of life. Finally, Scenarios C1–C2 refer to PE-based candle holders, where C1 involves landfill disposal and C2 integrates recycling.

LCA results show that Scenario A has the lowest environmental impact due to its simplicity and minimal processing. However, among candle holders, Scenario B3, produced without glycerol or shellac and incorporating recycling, proved the most sustainable, with lower environmental burdens than PE-based alternatives, particularly in global warming and fossil resource scarcity. Additionally, degradation tests showed that chestnut-based composites fully degraded within 120 days, with or without shellac, reinforcing their biodegradability and environmental benefits.

These findings highlight the potential of chestnut-based biocomposites as a circular economy solution, reducing reliance on fossil-based materials and mitigating plastic pollution. Future studies should focus on process optimization and industrial scalability to enhance the sustainability of these biomaterials.

Funding: This work received financial support from FCT/MCTES (UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020) through national funds and by the project Chestfilm, supported by “La Caixa” Foundation and Fundação para a Ciência e Tecnologia through the 4th edition of Promove program “O Futuro do Interior”. This work also received financial support from the PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

References:

- [1] A.O.C. Iroegbu, S.S. Ray, V. Mbarane, J.C. Bordado, J.P. Sardinha, ACS Omega, 6 (2021) 19343-19355.
- [2] S.B. Silva, O.M. Freitas, E.F. Vieira, C. Delerue-Matos, V.F. Domingues, Polym. Compos., 1 (2025) 1-20.



Phytochemical and biotechnological valorisation of winery by-products for prostate cancer cell modulation

Raquel Fernandes^{1,*}, Cláudia Botelho^{2,3}, Rúben Fernandes⁴, Ana Novo Barros^{1,*}

¹ Centre for Research and Technology of Agro-Environmental and Biological Sciences, CITAB, Inov4Agro, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

² CEB–Centre of Biological Engineering, University of Minho, Braga, Portugal

³ LABBELS–Associate Laboratory, University of Minho, Braga, Portugal

⁴ CECLIN, Centro de Estudos Clínicos, Hospital Fernando Pessoa, Gondomar, Portugal

* *rgfernandes@utad.pt (R.F.), abarros@utad.pt (A.N.B.)*

The increasing emphasis on waste reduction within sustainability and a circular economy has prompted the exploration of agricultural by-products as valuable resources. In the Douro region, over 20 million tonnes of winery by-products are generated annually, with most being discarded or used in low-value applications. This compromises the economic sustainability and competitiveness of the winery industry [1]. Data from our group identified grape stems as a rich source of polyphenols with promising pharmaceutical potential due to their antioxidant, anti-inflammatory, and anticancer properties. However, their effects on prostate cancer (PCa), the leading cause of cancer-related mortality in men remain largely unexplored.

In this study, polyphenols were extracted and characterized from four grape stem varieties representative of the Douro Region: Tinta Amarela, Sousão, Touriga Franca, and Touriga Nacional. Touriga Nacional exhibited the highest phenolic content, particularly proanthocyanidins and catechin derivatives, along with strong antioxidant activity. Consequently, the extract from this variety for selected for biological evaluation in PCa cells. The extract reduced reactive oxygen species (ROS), induced membrane disorganization, promoted G0/G1 cell cycle arrest, and increased necrosis in PCa cells, while having no impact on non-cancerous prostate cells.

This study highlights the dual benefits of mitigating the environmental footprint of the winery waste while providing sustainable innovative co-adjuvant therapies for PCa. Additionally, it supports economic and environmental sustainability by reducing waste management costs and enhancing the marketability of the winery industry through sustainable practices.

Acknowledgments: This work was supported by National Funds by FCT – Portuguese Foundation for Science and Technology, Ph.D. grant 2023.02608.BD and the projects UIDB/04033/2020 (<https://doi.org/10.54499/UIDB/04033/2020>), LA/P/0126/2020 (<https://doi.org/10.54499/LA/P/0126/2020>), and the strategic funding of the UIDB/04469/2020 unit. Additionally, support was provided by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020. The authors acknowledge CITAB – UTAD and CEB – UMinho for providing the necessary conditions for the development of this work and Rozès winery industry for supplying the grape stem varieties used in this study.

References:

- [1] R. Ferrer-Gallego, P. Silva, *Antioxidants*, 11 (2022) 2025.
- [2] Barros et al. *Food Research International* 2014 (2014) 375–384.
- [3] R. Fernandes, C. Costa, R. Fernandes, A.N. Barros. *Biomedicines* 11 (2023) 3140.

Pumpkin power: Turning butternut by-products into functional food gold

Elsa M Gonçalves^{1,2*}, Mafalda Silva^{1,3}, Arlette Nemputo³, Manuela Silva^{2,3}

¹INIAV-Instituto Nacional de Investigação Agrária e Veterinária, Unidade de Tecnologia e Inovação, Oeiras, Portugal.

² GeoBioTec-Geobiociências, Geoengenharias e Geotecnologias, FCT-UNL, Caparica, Portugal.

³FCT/UNL-Faculdade de Ciências e Tecnologia da Universidade NOVA de Lisboa, Caparica, Portugal.

* elsa.goncalves@iniav.pt

Agricultural by-products, often regarded as waste, are a valuable yet underutilized resource with potential applications in food and other industries [1,2]. Valorising these by-products addresses environmental challenges linked to waste disposal while promoting sustainable resource use and economic benefits. Butternut pumpkin (*Cucurbita moschata*), a widely cultivated vegetable, produces significant by-products like pulp, seeds, and peel during processing [3]. Rich in nutritional and bioactive compounds, the by-products hold promise for developing health-promoting foods [4].

This preliminary study aimed to evaluate the phenolic composition and antioxidant capacity of pumpkin pulp (P), seeds (S), and peel (C). Three independent samples of each by-product, obtained from the processing industry, were analysed. Methanolic extraction was performed to quantify total phenolic compounds (TPC, mg GAE/100 g fresh weight (fw)) and antioxidant activity (AOx) using DPPH (mg GAE/100 g fw) and FRAP (mmol FeSO₄•7H₂O/100 g fw) methods.

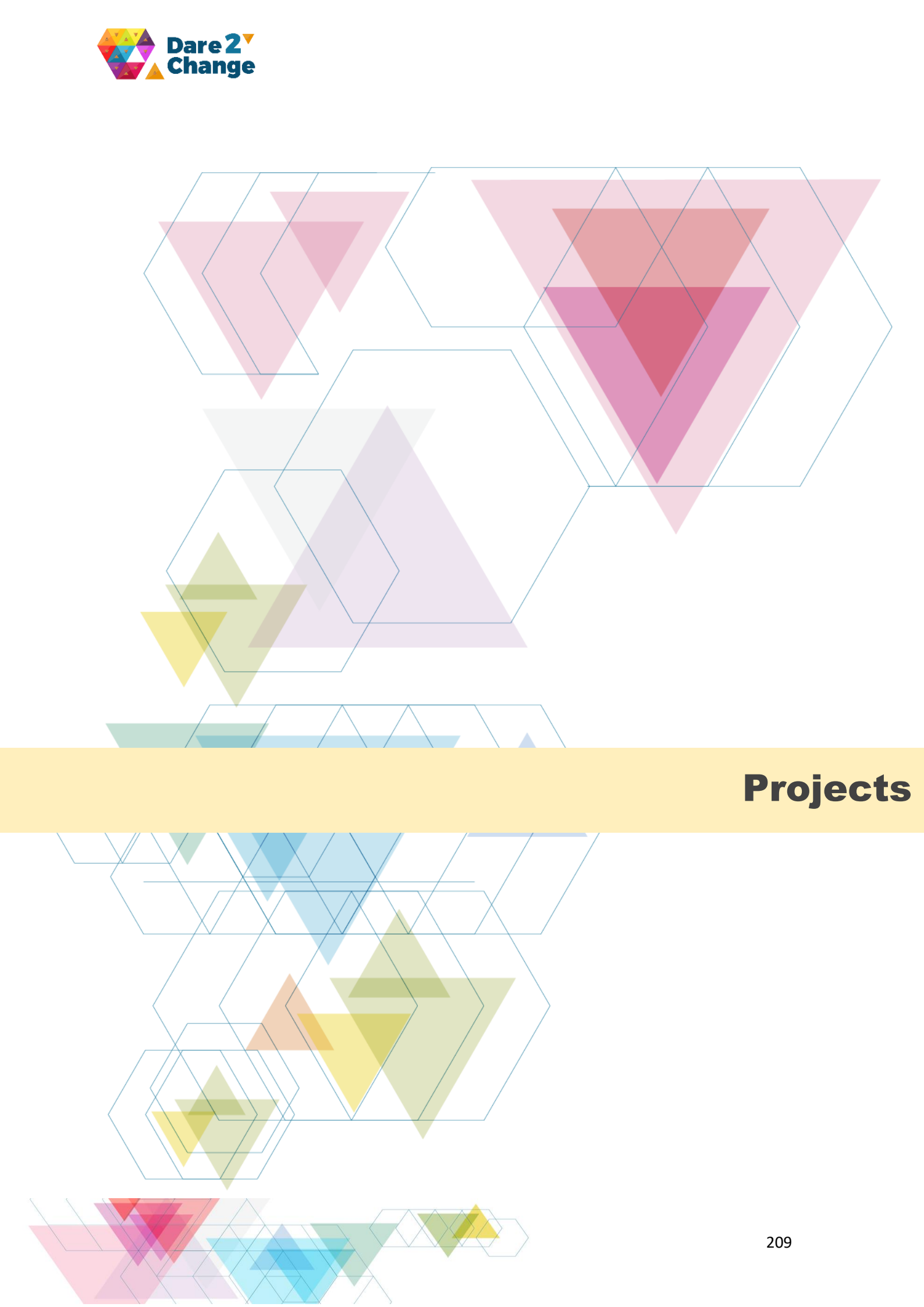
The results revealed considerable TPC concentrations in all the by-products, with values of 13.2 mg GAE/100 g fw for pulp, and 14.2 to 18.8 mg GAE/100 g fw for seeds and peel, respectively. The AOx activity, measured by DPPH, was 937 µmol TE/100 g fw for pulp but was significantly higher for seeds and peel, at 1253 and 1381 µmol TE/100 g fw, respectively. FRAP values were significantly lower, with no notable differences among the pumpkin parts. A strong correlation was observed between TPC and DPPH ($r = 1.00$; $p < 0.05$).

Traditionally discarded as waste, these by-products have shown significant potential as functional food ingredients, indicating that their valorisation could support more sustainable production practices and the creation of health-promoting food products. This study underscores the importance of utilizing pumpkin and its by-products in their entirety, providing innovative solutions to minimize waste while enhancing the nutritional and functional benefits of pumpkin-derived products.

Acknowledgments: This work was supported financially by the project UIDB/04035/2020 - GeoBioTec - Geobiosciences, Geoengineering, and Geotechnologies.

References:

- [1] Capanoglu, E.; Nemli, E.; Tomas-Barberan, F. Novel Approaches in the Valorization of Agricultural Wastes and Their Applications. *J. Agric. Food Chem.* **2022**, *70*, 6787–6804.
- [2] Hoehn, D.; Vázquez-Rowe, I.; Kahhat, R.; Margallo, M.; Laso, J.; Fernández-Ríos, A.; Ruiz-Salmón, I.; Aldaco, R. A critical review on food loss and waste quantification approaches: Is there a need to develop alternatives beyond the currently widespread pathways? *Resour. Conserv. Recycl.* **2023**, *188*, 106671.
- [3] Okuthe, G. Valorizing Fruit and Vegetable Waste: The Untapped Potential for Entrepreneurship in Sub-Saharan Africa—A Systematic Review. *Recycling* **2024**, *9*, 40.
- [4] Bas-Bellver, C.; Barrera, C.; Betoret, N.; Seguí, L. Turning agri-food cooperative vegetable residues into functional powdered ingredients for the food industry. *Sustainability* **2020**, *12*, 1284.



Projects

MICROORC: Orchestrating food system microbiomes to minimize food waste

¹ Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

² Nofima AS, Osloveien 1, NO-1430 Ås, Norway
**pcteixeira@ucp.pt*

The MICROORC project (<https://www.microorc.eu/>) aims to provide business innovations for monitoring and orchestrating the microbiome and by that reduce the amount of avoidable food waste – by developing tools, technologies, services, and guidelines for i) improved food process control, accurate shelf life prediction and dynamic date labelling, and ii) extension of shelf life by bioprotection and packaging technologies that control the growth of spoilage or pathogenic organisms. Five areas are selected for the potential for predicting and extending shelf life, reduction in food waste and fostering a sustainable food system:

- Predictive analytics models incorporating microbiome information to predict shelf life
- Time-temperature indicators (TTIs), sensing and smart label solutions for dynamic shelf life labelling
- Rapid detection assays for microbial indicators of food spoilage
- Microbiome based protection technologies to replace synthetic chemicals and increase shelf life and safety
- Novel packaging solutions targeting spoilage for sustainable development and increased shelf life

MICROORC will support the transition to more sustainable and healthy food systems by considering raw chicken and salmon as well as plant-based meat analogues. To guide and demonstrate the industrial, social and economic relevance of the MICROORC innovations, the project will evaluate consumer acceptance and the environmental sustainability aspects of all stages of the product life cycle, identify and help resolve legislative and regulatory challenges to implementation of new technologies, and propose a novel policy framework for microbiome control in the food system. A multinational, multi-actor and trans-disciplinary consortium including cutting-edge companies and experienced research institutions has been established to develop and pilot tools, technologies and guidelines that utilize and control microbiomes for fighting food waste to ensure transition to more sustainable food production and consumption. Portugal is represented by Universidade Católica Portuguesa, Vizelpas – Comércio de Artigos Plásticos Limitada, Lusiaves-Indústria e Comércio Agro-alimentar e Primor Charcutaria Prima.

MICROORC was funded by the European Union under Grant Agreement N° 101136248

FEAST | Food Systems that support transitions to healthy and sustainable diets

Center for Research and Development in Agrifood Systems and Sustainability (**CISAS**), Viana do Castelo, Portugal

Polytechnic Institute of Viana do Castelo (**IPVC**), Viana do Castelo, Portugal
ateresaoliveira@estg.ipv.pt; mvazvelho@estg.pt

The current European food system operates under a 'Lose-Lose-Lose-Win' model, where only large food corporations' benefit at the expense of the environment, public health, and the public sector. The project FEAST, Food Systems that support Transitions to Healthy and Sustainable Diets, aims to shift towards a 'Win-Win-Win-Win' food system by advancing research, innovation, and stakeholder-driven solutions that promote healthier and more sustainable diets across Europe.

Aligned with the EU Green Deal, Farm to Fork Strategy and the prioritisation of One Health approaches, FEAST adopts an interdisciplinary approach to co-design practical, scalable, and community-driven solutions. The project prioritizes behavioural dimensions of food system actors, often overlooked in policy and innovation, to ensure effective and lasting change. Crucially, FEAST is committed to a just and equitable transition, placing vulnerable groups at the centre to address health inequalities and empower all European citizens.

FEAST project aims to make it easy for every person in Europe to eat a delicious, healthier and more sustainable diet. To achieve this, FEAST, led by the University of Heidelberg, pursues four interlinked objectives: (1) identifying barriers and facilitators influencing dietary behaviour across diverse socio-economic and cultural contexts; (2) co-creating innovative tools and strategies to support informed food choices; (3) empowering individuals to adopt healthier and more sustainable dietary behaviours; and (4) boosting the adoption of evidence-based food and health policies through co-designed communication strategies and monitoring frameworks.

Over five years, FEAST will work collaboratively with food system stakeholders—including producers, distributors, retailers, and consumers—to drive systemic change and accelerate the transition to resilient, fair, and sustainable food systems in Europe.

Acknowledgments: FEAST is co-funded by the European Union's Horizon Europe research and innovation programme under grant agreement number 101060536 (**DOI:** 10.3030/101060536). Views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them (**Start date:** 1 July 2022; **End date:** 30 June 2027). The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to the CISAS, UIDB/05937/2020 (DOI: 10.54499/UIDB/05937/2020) and UIDP/05937/2020 (DOI: 10.54499/UIDP/05937/2020).

PAS-GRAS - De-risking metabolic, environmental and behavioral determinants of obesity in children, adolescents and young adults

Polytechnic Institute of Viana do Castelo, Avenida Atlântico 4900-348 Viana do Castelo, Viana do Castelo, Portugal

Center for Research and Development in Agrifood Systems and Sustainability (CISAS) of the Polytechnic Institute of Viana do Castelo, Avenida Atlântico 4900-348 Viana do Castelo, Viana do Castelo, Portugal

The project PAS-GRAS, led by the Center for Neuroscience and Cell Biology of the University of Coimbra (CNC-UC). “PAS-GRAS” involves partners from 8 European countries.

The EU-funded PAS-GRAS project aims to develop interdisciplinary research and innovation strategies that can change the paradigm of obesity prevention and treatment, providing practical solutions for healthy living in contemporary society through the prevention and reversal of obesity and associated metabolic problems, focusing on four critical age groups: prepubescent children (3-9 years), adolescents (10-18 years), young adults (19-25 years) and adults (25-55 years).

To this end, PAS-GRAS is structured around four fundamental pillars, firstly, the project will develop a personalized risk assessment tool (RAT), an algorithm designed to predict obesity onset and its complications, thereby enabling early intervention strategies. Secondly, it will design and implement RAT-based personalized interventions by integrating non-pharmacological lifestyle modifications, particularly Mediterranean Diet components, alongside pharmacological approaches targeting metabolic and neuroendocrine mechanisms. Thirdly, the initiative will engage and educate target groups through interactive digital tools, offering simplified RAT access and personalized diet and physical activity programs to promote sustainable behavioral changes. Finally, it will expand its reach across Europe by collaborating with healthcare centers, schools, sports clubs, municipalities, and public authorities to promote health and nutrition literacy, reinforcing a continent-wide campaign against obesity.

The anticipated outcomes include a significant reduction in overweight and obesity prevalence—15% in adults and 30% in children and adolescents by 2050. This effort aims to decrease obesity-related complications, reduce reliance on pharmaceutical and surgical interventions, and establish the Mediterranean Diet as a cornerstone for a healthier and more sustainable future. With a multidisciplinary research team spanning 12 top-tier universities and institutions, alongside contributions from civil organizations, patient groups, and industry partners, PAS-GRAS is set to make a lasting impact on obesity prevention and management in Europe.

Acknowledgements: To PAS-GRAS: DE-RISKING METABOLIC, ENVIRONMENTAL AND BEHAVIORAL DETERMINANTS OF OBESITY IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS. Grant agreement ID 101080329, DOI:10.3030/101080329 (starting date: 1 May 2023 and completion date: 30 April 2028) and to the Foundation for Science and Technology (FCT, Portugal) for its financial support via CISAS UIDB/05937/2020 <https://doi.org/10.54499/UIDB/05937/2020> and UIDP/05937/2020 <https://doi.org/10.54499/UIDP/05937/2020>.

Food Chase - Food supply-chain ecosystems for sustainability

Polytechnic Institute of Viana do Castelo, Avenida Atlântico 4900-348 Viana do Castelo, Viana do Castelo, Portugal

Center for Research and Development in Agrifood Systems and Sustainability (CISAS) of the Polytechnic Institute of Viana do Castelo, Avenida Atlântico 4900-348 Viana do Castelo, Viana do Castelo, Portugal

Food Chase - Food Supply-Chain Ecosystems for Sustainability, is an international cooperation project under the Erasmus+ programme, led by Cosvitec - University and Enterprise, involving partners from Portugal, Italy, Greece, Cyprus, Turkey, Romania, and Switzerland. This project is an European collaborative initiative dedicated to addressing the general issue of food loss and waste throughout the supply chain by fostering collaboration between key stakeholders, educational institutions, businesses, and policymakers. The aim of the project is to develop innovative solutions that promote sustainability in the agri-food sector.

Key objectives include establishing collaborative networks to innovate food supply chain training, developing a comprehensive model that defines competencies required to reduce waste, to standardize food loss reduction efforts and introducing a specialized curriculum to create the Farm-To-Fork Specialist role. This framework informs the development of a specialized training curriculum designed to upskill professionals and introduces the Farm-to-Fork Specialist role by bridging knowledge gaps and providing targeted training. These efforts aim to transform educational systems and offer new job opportunities in the agri-food sector.

The project anticipates a multi-level impact across the agri-food sector, driving structural changes, introducing innovations in education and business systems, and supporting improvements in agri-food businesses by integrating project methodologies and outputs into regular activities, partner organizations aim to foster collaboration with diverse actors, ultimately enhancing sustainability in the food industry.

Beyond education, Food Chase supports structural innovation in food supply chains, encouraging systemic change and policy recommendations. The project's long-term impact includes improved resource efficiency, reduced environmental footprint, and a more sustainable food economy across Europe.

Acknowledgements: To FOODCHASE - Food Supply-Chain Ecosystems for Sustainability. ERASMUS-EDU-2023-PI-ALL-INNO Grant agreement ID101140250 (starting date: 01 February 2024 and completion date: 31 January 2027) and to the Foundation for Science and Technology (FCT, Portugal) for its financial support via CISAS UIDB/05937/2020 <https://doi.org/10.54499/UIDB/05937/2020> and UIDP/05937/2020 <https://doi.org/10.54499/UIDP/05937/2020>.

