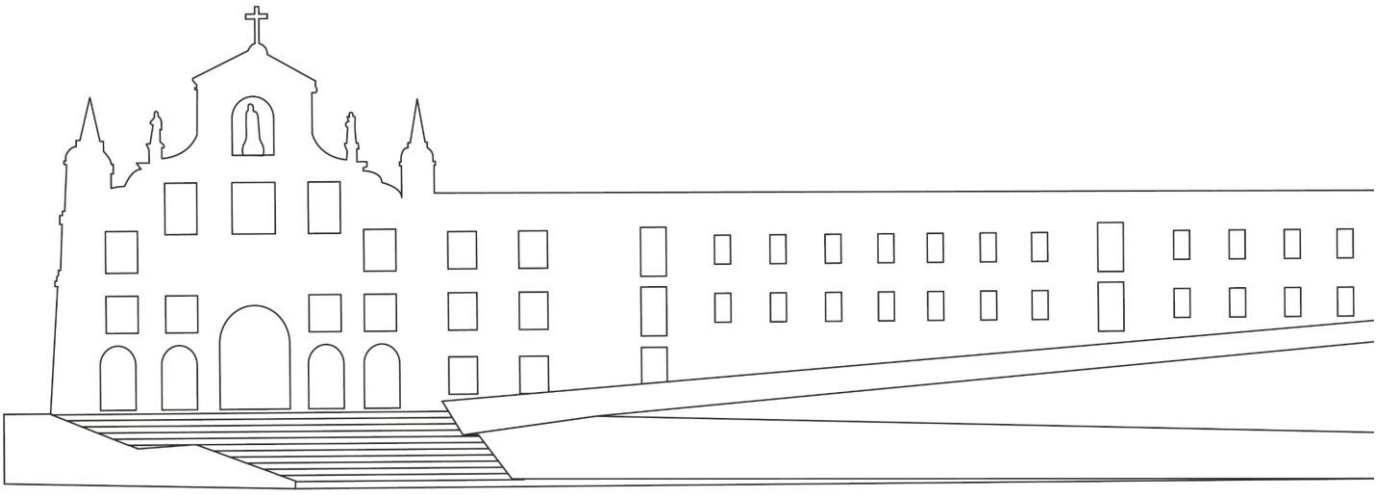
XVIII Encontro de Química dos Alimentos

Coimbra | 14 - 16 de outubro'26

Livro de Resumos



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PREFÁCIO

É com particular satisfação que apresentamos o **Livro de Resumos do XVIII Encontro de Química dos Alimentos — XVIII EQA**, que reúne os contributos científicos apresentados nesta edição e constitui, simultaneamente, mais um capítulo da história de uma comunidade científica que há décadas encontra neste evento um espaço privilegiado de partilha, discussão e colaboração.

Os Encontros de Química dos Alimentos (EQA) ocupam hoje um lugar reconhecido na comunidade científica portuguesa ligada aos alimentos. A sua longevidade e continuidade são, por si só, testemunho da importância de uma iniciativa que soube acompanhar a evolução da ciência, da tecnologia e da sociedade, mantendo sempre como propósito fundamental a aproximação entre **investigação, conhecimento, inovação e aplicação**.

Mas uma história com esta dimensão tem necessariamente os seus fundadores e aqueles a quem devemos o primeiro impulso.

Neste XVIII EQA queremos, por isso, prestar uma homenagem muito especial ao **Professor Doutor António Empis**, reconhecendo o seu papel, juntamente com o **Professor Doutor Justino**, na fundação dos Encontros de Química dos Alimentos.

A criação destes encontros resultou de uma visão que permanece extraordinariamente atual: a de que o desenvolvimento da ciência dos alimentos exige espaços de encontro onde investigadores de diferentes áreas possam apresentar resultados, confrontar ideias, estabelecer colaborações e, sobretudo, construir uma comunidade.

O Professor José Empis, docente e investigador do sistema de ensino superior universitário e o Professor Jorge Justino, docente e investigador do sistema de ensino superior politécnico, compreenderam, desde cedo, que o progresso científico não se faz apenas através do trabalho individual ou institucional. Faz-se também através da **partilha e da criação de redes de conhecimento**.

Os EQA nasceram dessa visão (consultar a Linha do tempo dos 18 Encontros de Química dos Alimentos).

E o facto de chegarmos hoje à sua décima oitava edição é a melhor demonstração da vitalidade dessa ideia.

Ao longo das diferentes edições, os EQA acompanharam a profunda transformação da ciência dos alimentos. Novos métodos analíticos, novas tecnologias de processamento, novos conhecimentos sobre composição e funcionalidade dos alimentos, novas preocupações com a segurança alimentar, a saúde, a sustentabilidade e o desperdício foram sucessivamente incorporados na agenda científica destes encontros.

Mudaram as perguntas, mudaram as ferramentas e mudaram os desafios.

Permaneceu, porém, a necessidade de nos encontrarmos.

É essa continuidade que este XVIII EQA procura celebrar.

O tema escolhido para esta edição — **"Mais de 10 000 anos de invenções alimentares: diálogo entre investigação, indústria e mercados"** — convida-nos a olhar simultaneamente para o passado e para o futuro.

Para o passado, porque a história da alimentação é também uma história de inovação. Desde as primeiras formas de conservação e transformação dos alimentos até às tecnologias mais avançadas dos nossos dias, o ser humano tem procurado compreender e transformar aquilo que come.

Para o futuro, porque os desafios que hoje enfrentamos exigem novas respostas científicas e tecnológicas. Segurança alimentar, saúde, sustentabilidade, eficiência no uso dos recursos, redução do desperdício, valorização de subprodutos e adaptação às alterações climáticas são apenas alguns dos desafios que exigem conhecimento e colaboração.

É neste contexto que o diálogo entre investigação, indústria e mercados assume particular importância.

A investigação produz conhecimento e abre novas possibilidades.

A indústria transforma conhecimento em processos, produtos e soluções.

E os mercados, juntamente com os consumidores e a sociedade, colocam novas necessidades e novos desafios.

A aproximação destas diferentes dimensões é essencial para que o conhecimento produzido nos nossos laboratórios possa contribuir efetivamente para uma transformação positiva dos sistemas alimentares.

O presente Livro de Resumos é, nesse sentido, mais do que uma compilação de trabalhos científicos. É o retrato, ainda que necessariamente parcial, da vitalidade de uma comunidade.

Nas suas páginas encontramos diferentes gerações de investigadores, diferentes instituições, diferentes áreas científicas e diferentes abordagens. Encontramos ciência fundamental e investigação aplicada, novas metodologias e novas perguntas, resultados consolidados e caminhos ainda em construção.

Cada resumo representa trabalho.

Representa tempo, dedicação e discussão.

E representa também a vontade de partilhar conhecimento com os outros.

É precisamente essa cultura de partilha que devemos preservar.

E é por isso que a homenagem póstuma ao Professor José Empis, neste XVIII EQA, assume um significado especial.

Ao homenageá-lo, homenageamos também o Professor Jorge Justino e todos aqueles que participaram nos primeiros passos desta iniciativa.

Homenageamos uma geração que acreditou na importância de criar uma comunidade. E reconhecemos que aquilo que hoje temos só foi possível porque alguém, há 33 anos, teve a visão de começar.

Aos fundadores devemos a memória. Às gerações que se seguiram devemos a continuidade. Às gerações presentes cabe-nos a responsabilidade de construir o futuro.

É nesta perspetiva de continuidade que devemos olhar para os EQA.

Não como uma sucessão de encontros isolados, mas como uma história coletiva que se vai construindo de edição em edição, através das pessoas que participam, das ideias que partilham e das colaborações que estabelecem.

Este XVIII EQA é, por isso, simultaneamente, um ponto de chegada e um ponto de partida.

Um ponto de chegada, porque celebra uma história longa e rica.

Um ponto de partida, porque cada nova geração traz consigo novas perguntas, novas ferramentas e novas possibilidades.

Que este Livro de Resumos possa preservar uma parte dessa história.

Que possa servir de memória dos trabalhos apresentados nesta edição.

E que, sobretudo, possa ser visto como um testemunho de uma comunidade científica que continua a acreditar no valor do conhecimento partilhado e na importância de trabalhar em conjunto.

Ao Professor Doutor António Empis e ao Professor Doutor Jorge Justino, deixamos o nosso reconhecimento e a nossa gratidão pela visão que esteve na origem dos Encontros de Química dos Alimentos.

A todos os autores, conferencistas, participantes, membros das comissões científica e organizadora, parceiros e instituições que tornam possível esta edição, deixamos igualmente o nosso profundo agradecimento.

E a todos os que participam neste XVIII EQA, desejamos que encontrem nestes dias conhecimento, inspiração, debate e novas oportunidades de colaboração.

Que continuemos, juntos, a escrever esta história.

Coimbra, outubro de 2026

As responsáveis da Comissão Organizadora do XVIII EQA

Aida Moreira da Silva e Maria João Barroca

Linha do tempo dos 18 Encontros de Química dos Alimentos*: da primeira edição, em Santarém, à edição de 2026, em Coimbra*.

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Base histórica: Livros de Resumos do XVI EQA (1993–2022); páginas oficiais do XVII e XVIII EQA.

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FERMENTAÇÕES: DA TRADIÇÃO À FERMENTAÇÃO DE PRECISÃO



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A sua atividade de investigação centra-se na história da indústria conserveira portuguesa, com particular enfoque nas suas empresas, marcas e embalagens, sendo responsável pelo projeto e museu digital conservasdeportugal.com. Desde 2018, desenvolve também investigação sobre garum, tendo realizado diversas experiências no âmbito do projeto Selo de Mar, em colaboração com as Ruínas Romanas de Tróia, o Instituto Superior de Agronomia da Universidade de Lisboa e a investigadora britânica Sally Grainger.

O seu trabalho caracteriza-se pela articulação entre investigação histórica, cultura material, design e património alimentar, explorando as relações entre produção, identidade, memória e cultura alimentar.



- Substituida por Joana Sales

Engenheira Alimentar e doutorada em Engenharia Agronómica pelo Instituto Superior de Agronomia da Universidade de Lisboa. Atualmente, exerce funções como Técnica Superior na mesma instituição, desenvolvendo atividade nas áreas da ciência dos alimentos, bioquímica, sustentabilidade e inovação agroalimentar, com intervenção em investigação, apoio técnico e projetos de investigação e desenvolvimento.

Possui também experiência de docência no ensino superior, nomeadamente nas áreas da análise química de alimentos, técnicas laboratoriais, química e bioquímica. Tem participado em diversos projetos de investigação nacionais e internacionais, desenvolvendo um percurso de investigação aplicada e de colaboração multidisciplinar, incluindo parcerias com empresas. É autora e coautora de publicações científicas, livros e capítulos de livros, bem como de comunicações orais e em painel apresentadas em encontros científicos nacionais e internacionais

Lusitanian Garum: from history to scientific rediscovery

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Garum was one of the most important food products of the Roman world, with Lusitania playing a prominent role in the production and export of fish-based preparations. Among the various industrial complexes identified, Tróia stands out as a paradigmatic example and is considered the largest known fish-processing centre in the Roman Empire[1]. Despite its historical and economic significance, knowledge of Garum production methods remained incomplete for centuries due to the scarcity and fragmentary nature of the available historical sources.

This study presents the work undertaken by Selo de Mar, in collaboration with archaeologists, historians, chemists and food engineers, to recreate Lusitanian Garum by reproducing, as closely as possible, the production conditions used in Antiquity while simultaneously ensuring compliance with modern food safety requirements.

Following several Garum production experiments using different fish species, which provided practical experience and helped refine production procedures, it became possible to move towards a new phase of experimentation. In 2021, a Garum production trial was conducted at the Roman Ruins of Tróia, in an original processing tank within the archaeological complex, using sardines (*Sardina pilchardus*) and salt from the Sado Estuary. This experiment made it possible to monitor the fermentation process over several months and obtain an unprecedented set of scientific data on its progression. The aim was to establish a scientific basis for recreating a product whose historical production relied on highly complex empirical knowledge.

Within this framework, the physicochemical, nutritional and sensory evolution of different Garum formulations, produced from various fish species and using different production methods, was characterised[2].

In sardine Garum, sardines being one of the species most widely used at Tróia, the progressive formation of compounds associated with umami taste, particularly glutamate, was observed, reaching concentrations exceeding 5 mg/g after fermentation. This was accompanied by an increase in organic acids and nitrogenous compounds resulting from proteolysis. The studies also confirmed microbiological stability and highlighted the characteristic mineral richness of this type of fermented product.

The project also involved the development of innovative Garum formulations derived from non-fish sources, using bee pollen, hibiscus and macroalgae (*Fucus vesiculosus*, *Palmaria palmata* and *Codium*) as raw materials. These new formulations exhibited distinct fermentation pathways, with differentiated profiles of organic acids, antioxidant capacity, mineral composition and glutamate content. The results highlight the potential of Garum production technology as a platform for adding value to diverse biological resources and developing new sustainable food ingredients, thereby opening up opportunities for flavour diversification and innovative sensory experiences.

The combination of historical heritage, fermentation science and food innovation demonstrates that Garum can be understood not only as a legacy of the past but also as a valuable source of knowledge for the development of foods of the future.

Keywords: Lusitanian Garum; fermentation; food heritage; umami; food innovation.
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Doutorada em Engenharia Alimentar pelo Instituto Superior de Agronomia da Universidade de Lisboa, onde exerce funções de docente desde 2013 nas áreas dos Alimentos Fermentados e da Microbiologia Funcional. A sua atividade científica combina conhecimentos de biologia molecular e fisiologia microbiana com a área das fermentações, com particular enfoque no desenvolvimento de novos produtos alimentares fermentados. A sua investigação tem incidido no desenvolvimento e produção de alimentos e bebidas fermentados a partir de leguminosas subvalorizadas e de subprodutos da indústria alimentar, procurando aumentar o seu valor económico, qualidade nutricional e segurança. Desenvolve também investigação dedicada à criação de culturas iniciadoras mais seguras para a produção de alimentos e bebidas fermentados

Fermentation as a tool for food loss reduction and sustainable product innovation

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Food loss is a major challenge for the sustainability of current agro-industrial food systems, representing an inefficient use of raw materials, water and energy and contributing to significant environmental impacts. At the same time, food production generates surplus materials and processing side-streams that retain nutritive and health value but are often underutilised. Strategies that preserve, transform and reintegrate these resources into the food chain are essential for the transition towards more circular food systems while meeting consumers' demand for effective waste-reduction strategies.[1]

Fermentation offers a flexible approach to this challenge. It has long been used to extend a product's shelf life, improve food safety, and enhance flavour, texture, and nutritional qualities. It can also be seen as a biotechnological means of converting underused resources into safe, stable, and appealing ingredients and products. Through microbial metabolism, the complex structure of foods can be altered, undesirable compounds reduced, nutrients and bioactive molecules released or transformed, and metabolites with technological, sensory, or functional value produced. Ongoing advances in microbial ecology, starter culture design, and analytical technologies are gradually moving fermentation from a purely empirical method of preservation to a platform for sustainable food innovation.[2]

The lecture will examine this shift by drawing on examples from plant materials, little-used crops, marine resources, and food-industry by-products. The fermentation of surplus and off-specification agricultural vegetables and fruits, as well as macroalgae, shows how perishable raw materials can be turned into novel foods while also enabling wider use of them. Adapting traditional methods such as miso and tempeh to underused legumes, for example, grass pea and lupin, demonstrates that fermentation has the potential to promote agrobiodiversity and more sustainable cropping systems, and open up new possibilities for crops well-suited to local conditions, while providing useful principles for contemporary strategies to maximise the use of food resources. Particular attention will be given to the valorisation of food-industry side streams and to the use of other fermented products to produce functional ingredients or naturally derived preservation systems. In this context, fermentation provides a route from waste prevention to value creation: rather than simply treating a residue, microbial metabolism can redesign it as a new food resource.[2]

The successful implementation of these strategies nevertheless requires consideration of microbial safety, stability, sensory quality, nutritional value and process reproducibility. Understanding microbial succession and metabolic interactions becomes particularly important when moving from spontaneous fermentation towards defined microbial consortia. The integration of conventional microbiology with metagenomic, metabolomic and physicochemical approaches provides new opportunities to identify the microorganisms and metabolic pathways associated with desirable outcomes and to design safer and more predictable fermentation processes.[3]

The role of fermentation in reducing food loss therefore lies in the area where tradition meets precision. Traditional fermented foods offer remarkable microbial diversity, along with diverse processes and practical knowledge, while modern methods enable the selection and control of microorganisms, microbial communities, and fermentation conditions to meet specific technological aims.

Ultimately, fermentation can promote a change in perspective: surplus materials, food side-streams and underused biological resources should not be regarded as waste, but as substrates whose potential remains to be unlocked. By connecting traditional knowledge with microbial ecology, food chemistry and biotechnology, fermentation provides a bridge between ancient food practices and future circular food systems, enabling us not only to preserve food, but also to preserve resources, create value and develop innovative and sustainable products.[3]

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Evolution of the volatile compound profile during the fermentation of garum produced from Pollen, Hibiscus, and Fucus

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Garum is one of the oldest known fermented condiments, traditionally produced by fermenting fish viscera in brine and valued for its distinctive sensory profile[1]. In recent years, the increasing demand for plant-based alternatives has driven the development of new products inspired by traditional garum. In this context, Selo de Mar developed three innovative garum formulations: fresh pollen garum (fresh pollen, water, and salt), hibiscus flower garum (hibiscus flower, water, salt, koji, and pumpkin seeds), and Fucus vesiculosus garum (Fucus vesiculosus, water, salt, and koji).

The characterization of the volatile profile is essential for understanding the evolution of fermentation and the production of aroma-related compounds that influence sensory properties. Therefore, the volatile profiles of the three garum were analyzed by solid-phase microextraction (SPME) using a DVB/CAR/PDMS fiber, followed by gas chromatography coupled to mass spectrometry (GC-MS), with separation on an HP-5 MS capillary column.

Despite the use of different raw materials, all three garum formulations showed a similar overall evolution of their volatile profiles throughout fermentation. The garum formulations were fermented in sealed plastic buckets and incubated in a temperature-controlled chamber at 30–40 °C for 130 days. As an example, Figure 1 presents the chromatogram obtained for the fresh pollen garum.

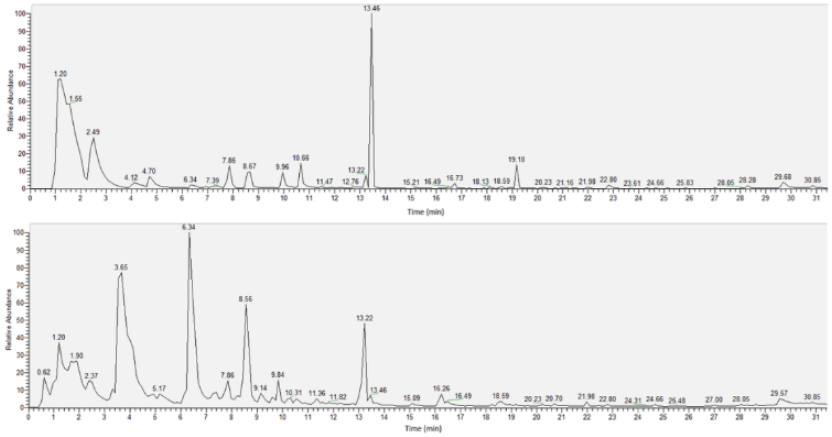


Figure 1 - Representative chromatograms of pollen-based garum at day 0 and day 130 of fermentation, respectively, obtained by SPME-GC-MS.

At the initial stage of fermentation, alcohols such as ethanol and 3-methyl-1-butanol, associated with malty and fermentative notes, predominated. After 130 days of fermentation, a change in the volatile profile was observed, characterized by a decrease in alcohols and an increase in aromatic aldehydes, such as benzaldehyde and furfural, which are commonly associated with bitter almond and caramel notes, respectively2. This evolution may be related to sugar degradation and the transformation of phenolic compounds by Lactic Acid Bacteria and halophilic Archea3.

In addition to this common progression, differences were observed among the three garum formulations. The Fucus sp. garum showed higher acetic acid content at the intermediate stage of fermentation, whereas the pollen and hibiscus garum exhibited a higher abundance of furfural at the end of fermentation.

These findings highlighted the potential of different raw materials to produce unique aroma profiles, thereby expanding the sensory diversity and gastronomic value of garum-inspired products.

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Scalable Mycoprotein Production by Pleurotus ostreatus through Valorization of Brewer's Spent Grain in Solid-Submerged Fermentation

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The growing demand for alternative protein sources has driven increasing interest in microbial proteins, such as mycoprotein, due to their lower environmental footprint and high nutritional value. In this context, the use of agro-food by-products as fermentation substrates in solid-submerged fermentation (SSmF) represents a valuable opportunity to enhance circularity and resource efficiency within food systems. However, challenges related to process optimization, scalability, and safety still remain, limiting large-scale industrial implementation. Therefore, the main objective of this study was to develop a scalable and efficient SSmF process for mycoprotein production using filamentous fungi and agro-food by-products.

Initially, five edible fungal species were screened under submerged cultivation conditions in shake flasks to evaluate biomass production and mycoprotein yields. The selected strain, Pleurotus ostreatus s1, was subsequently cultivated in media supplemented with 10% (w/v) of brewer's spent grain (BSG), cocoa bean shell (CBS), peanut peel (PP), and banana peel (BP) to assess growth performance on these by-products. The bioprocess was then scaled to stirred-tank and airlift bioreactors operating under controlled conditions with a working volume of 2.5 L. Biomass production and mycoprotein yield were used as key performance indicators to evaluate process efficiency across the different cultivation systems.

The results demonstrated that P. ostreatus s1 was the most suitable fungal species, combining high biomass production (16.02 ± 2.80 g/L) with a consistent mycoprotein yield (8.72 ± 0.35 g/100 g). Among the tested by-products, BSG supported the highest mycoprotein content (14.22 ± 0.95 g/100 g). Notably, both bioreactor configurations outperformed flask fermentations in terms of biomass productivity, demonstrating the positive impact of improved mixing and oxygen transfer conditions. The stirred-tank bioreactor achieved 30.52 ± 10.75 g/L of biomass, with a mycoprotein yield of 10.54 ± 1.55 g/100 g, while the airlift bioreactor reached 22.00 ± 5.12 g/L of biomass, with a mycoprotein yield of 12.23 ± 0.15 g/100 g. Scale-up experiments confirmed that both bioreactor configurations are suitable for the cultivation of P. ostreatus under SSmF conditions. These findings indicate that the process is robust, scalable, and adaptable, offering flexibility for future industrial implementation. In terms of safety, mycotoxin analysis revealed that fumonisin B2 (41.4 µg/kg) was the only mycotoxin detected in both the original BSG and the fermented biomass. Notably, its concentration decreased by 25% following fungal fermentation, demonstrating the potential of the process to mitigate contamination risks associated with agro-food by-products.

Overall, the results obtained validate the feasibility of producing mycoprotein from P. ostreatus through the valorization of BSG, supporting the transition toward a more sustainable and circular source of protein.

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Development and physicochemical characterization of a potential synbiotic food (kefir) with the incorporation of Arbutus Unedo l.: a biotechnological approach for the reduction of added sugars

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Fermented foods, specifically synbiotics, are increasingly establishing themselves in the food industry, highlighting their contribution to human health1. The strawberry tree fruit (Arbutus unedo L.), endemic to the Mediterranean region, is easily fermentable due to its high content of sugars, and notable for its content in dietary fibre, phenolic compounds, and strong antioxidant potential2,3. These characteristics confer it an excellent prebiotic profile.

This study aimed to develop a fermented potential synbiotic beverage (milk kefir) using pasteurised Arbutus unedo L. fruit paste for the partial or total substitution of added sucrose, thereby promoting the reduction of simple sugars.

Five formulations were prepared (with sugar substitution rates of 0%, 25%, 50%, 75%, and 100%) using whole UHT milk inoculated with a complex mesophilic kefir culture, incubated at 25 ºC. Physicochemical characteristics were determined using a TA-XTplus texturometer, FoodScan™ rapid analyser for protein, fat, and total solids, Atago Palette PR-32 digital refractometer for degrees Brix, TitroMatic Crison automatic titrator for pH and acidity, and Rotronic HygroPalm HP23-AW portable digital hygrometer for water activity. Sensory analysis was conducted with an informed panel using preference ranking tests, flavour profile, focus groups, and stability tests. Microbiological analyses were performed using the automated commercial system TEMPO®. After analysis, the most promising formulation was selected. For this selected formulation, real-time shelf life was determined through physicochemical, microbiological, and sensory analyses.

The physicochemical evaluation indicated that the increase in Arbutus unedo L. led to a progressive decrease in total soluble solids (ºBrix) at the end of fermentation. The fermentation kinetics were not affected by the addition of the fruit's, with optimal values at 24 hours. Rheological analysis evidenced the maintenance of the matrix's structural integrity regarding firmness, consistency, and viscosity index, with no statistically significant differences observed ($p > 0.05$). The 50 % formulation showed the highest nominal cohesiveness (-0.0933 N). The progressive incorporation of the fruit caused a gradual reduction in proteins, fats, and total solids in the different formulations; Despite changes in total solids and protein content, the viscosity and consistency remained stable. This highlights the potential technological and functionality of medronho fibers as stabilizing or structuring agents. Water activity did not differ significantly between formulations. Subsequently, the physicochemical, sensory, and economic evaluation revealed that the most promising formulation was the 25% substitution with strawberry tree fruit.

The kefir meets microbiological count according to the current Portuguese regulations, and demonstrated microbiological stability for 21 days under refrigeration.

The incorporation of 25% strawberry tree fruit technologically enables reduction of added sugar, and the production of an innovative, and potential synbiotic food, and adding value to an underutilized wild resource.

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Fermented Pulse-Based Cheese Alternatives: Applying Traditional Fermentation to Next-Generation Protein Foods

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Fermentation has accompanied human food innovation for millennia, playing a central role in the development of cheese and other fermented foods[1]. Today, this ancient technology is being revisited to address emerging challenges associated with sustainability, nutrition, and the growing demand for alternative protein sources[2]. Plant-based cheese alternatives (PBCA) represent a rapidly expanding market; however, reproducing the nutritional, structural, and sensory characteristics of dairy cheese remains a significant challenge.

This study explored the use of fermentation as a tool to enhance pulse-based PBCA produced from chickpea, yellow pea, and chickpea–lupin matrices. The effects of fermentation and curing were evaluated through physicochemical characterization, texture profile analysis, protein digestibility assessment, SDS–PAGE protein profiling, and volatile compound analysis by GC–MS.

The developed PBCA (Figure 1) exhibited high protein contents (18–20%), surpassing values typically reported for commercial plant-based cheeses. Chickpea-based formulations displayed texture characteristics comparable to those of soft and semi-hard cheeses, while in vitro protein digestibility ranged from 70 to 100%. Protein profiling confirmed the preservation of the main pulse protein fractions throughout processing. More than 40 volatile compounds were identified, including ketones, aldehydes, alcohols, and short-chain fatty acids associated with characteristic fermented and cheese-like aromas. Fermentation significantly modified the volatile profile, promoting the generation of compounds commonly associated with cheese ripening.

The results demonstrate that pulse fermentation can contribute to the development of nutritionally rich and sensorially improved cheese alternatives, highlighting the potential of combining traditional food biotechnologies with alternative protein sources. This work illustrates how knowledge generated through food chemistry and fermentation science can support the creation of innovative products capable of responding to current consumer demands and future market opportunities.



Figure 1 - Two examples of PBCA development with chickpea and lupin formulation, that have gone through the fermentation and curing process.

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Boosting the Nutritional Potential of Vegetable Co-Products via Lactic Acid Fermentation

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Food loss and waste are increasingly recognized as major environmental, economic, and social challenges, particularly regarding the disposal of nutrient-rich and edible vegetable co-products generated throughout the food chain. Although many of these materials retain significant nutritional value, their high perishability and limited industrial application often result in underutilization. In this context, lactic acid fermentation has emerged as a promising processing strategy to stabilize vegetable co-products while simultaneously improving their nutritional and functional value1.

In the present work, Portuguese cole stems and old leaves, carrot leaves, and mixtures of broccoli leaves with scrap carrots were selected as promising vegetable co-products for lactic acid fermentation, transformed into pastes, and processed at pilot scale using previously optimized lactic acid bacteria consortia. Both non-fermented (raw) and fermented materials were characterized regarding nutritional composition and potentially bioactive properties. Proximate composition analyses were performed following AOAC methods. Mineral composition was assessed for both raw and fermented vegetables, but also for products after in vitro digestion by Infogest protocol2. Digested and undigested fractions were compared to assess the bioacessibility of minerals in the raw and fermented products. Furthermore, bioactive-related parameters such as total phenolic compounds, antioxidant activity (DPPH and FRAP assays), ascorbic acid, and chlorophylls content were evaluated before and after fermentation. Additionally, raffinose content was estimated by HPLC, to assess the impact of fermentation on this oligosaccharide associated with bloating and flatulence3.

Overall, the fermentation process promoted compositional and functional changes that depended on the raw material and fermentation consortium used. In general, fermented products showed increased phenolic compound content and antioxidant activity, ranging from 1.5- to 6.4-fold depending on the extraction solvent used. Regarding raffinose, non-fermented broccoli presented concentrations of approximately 250 mg/100g, while fermentation reduced this content by around 70%, supporting the potential of LAB fermentation to improve digestibility and consumer acceptance of this product. Differences between raw materials highlighted the importance of substrate composition in fermentation performance and final product characteristics.

The obtained results reinforce the potential of controlled lactic acid fermentation as a sustainable strategy for the valorization of vegetable co-products into stable, nutritionally relevant, and potentially functional food ingredients. Beyond reducing food loss, fermentation may contribute to the development of innovative plant-based products with added nutritional and technological value.

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Influence of Starter Cultures and Flavourings on the Sensory and Physicochemical Optimisation of Set-Style Flavoured Yoghurts

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This study focused on optimising key sensory and physicochemical factors, including texture, gel strength, pH, and acidity, by testing various starter cultures and flavourings. The experimental work involved pilot-scale fermentations. To better understand the structural and compositional differences among the tested cultures, vibrational spectroscopy (FTIR-ATR) was used.

A factorial ANOVA was conducted using starter culture type and flavouring as fixed factors. This statistical approach made it possible to quantify not only the individual effects of cultures and flavourings but also their interactions on the targeted quality attributes. Although the culture type was identified as the most influential factor, particularly for gel formation and texture, the analysis also showed significant effects of flavourings and how their interactions with cultures lead to product variability.

To complement the formulation study, a 56-day shelf-life evaluation was performed on an industrial scale. The results confirmed that the influence of starter cultures becomes increasingly pronounced during storage, affecting long-term gel stability and consistency. Flavourings, though less dominant, contributed to differences in perceived acidity and the overall sensory profile.

The optimised formulation developed in this work demonstrated superior performance compared to the existing product, showing enhanced structural stability, improved sensory acceptance, and greater technological suitability. Altogether, the findings underscore the importance of integrating culture selection, flavour choice and statistical modelling in the development of high-quality flavoured yoghurts, providing valuable guidance for future product innovation in an industrial context.

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Freezing-Assisted Fermentation of *Gracilaria gracilis* and *Codium tomentosum*: From Process Optimization to Nutritional and Antioxidant Characterization

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The growing demand for sustainable and health-promoting food ingredients has increased interest in macroalgae as nutrient-rich biomass with considerable functional potential. However, their use as food ingredients remains constrained by the limited accessibility of nutrients trapped within the complex and recalcitrant seaweed matrix[1]. Addressing this challenge requires innovative bioprocessing strategies that support the development of sustainable food systems. Fermentation has emerged as a promising approach to overcome these limitations while enhancing the nutritional and functional value of macroalgae. Gracilaria gracilis (red alga) and Codium tomentosum (green alga) are commercially available and widely recognized in Portugal. Both species are known for their functional polysaccharides, bioactive compounds and high antioxidant potential[2,3].

This study investigated the integrated optimisation of pre-treatments and fermentation conditions for G. gracilis and C. tomentosum to enhance substrate accessibility and enable controlled bioconversion into value-added food products. Pre-treatment optimization involved evaluating rehydration time, water retention capacity, ultrasonication conditions and particle size reduction to establish optimal fermentation conditions by enhancing nutrient availability and promoting cell wall hydrolysis. Fermentation was conducted using lactic acid bacteria (a consortium of Lactococcus and Streptococcus, or Streptococcus thermophilus alone) and Aspergillus oryzae (koji). Ultrasonication treatment, freezing effect, and the effect of adding acetic acid were evaluated (Figure 1).

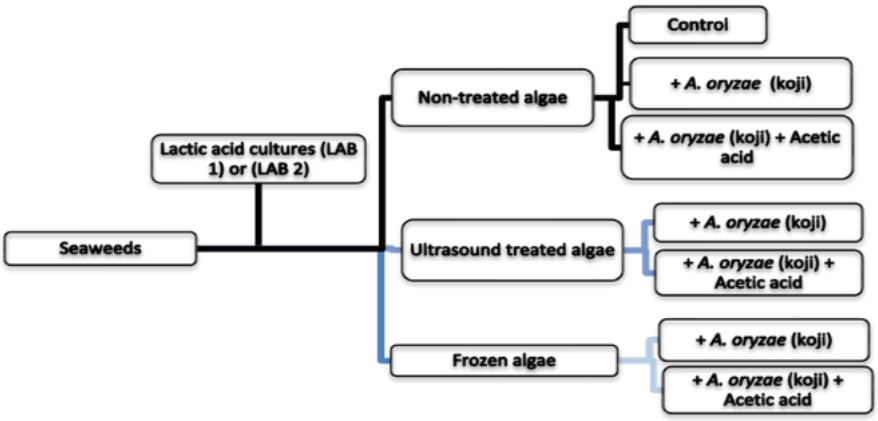


Figure 1 - Tested conditions workflow in seaweed fermentation.

The fermentation process was monitored for pH, total soluble solids content (TSS, oBrix), substrate consumption and metabolite production (by HPLC) every 24 hours until stabilization. The optimal fermentation strategy, consisting of freezing pre-treatment, koji supplementation, acetic acid acidification and S. thermophilus monoculture, was selected and successfully applied to scale-up fermentations (500 mL). The fermented macroalgal pastes were characterized for physicochemical stability, texture, nutritional composition and antioxidant activity.

The results demonstrated distinct species-dependent structural behaviour between G. gracilis and C. tomentosum, highlighting the need for species-specific optimization of biomass processing to maximize substrate accessibility and hydrolysis efficiency. Across both species, the selected conditions promoted greater soluble solids release (Δ°Brix), enhanced fermentability and increased metabolic precursor availability, yielding glucose concentrations that consistently exceeded those obtained from both ultrasound-treated and untreated biomass. These conditions also accelerated acidification to microbiologically relevant pH values (≈3.6–3.8 for G. gracilis and ≈4.6 for C. tomentosum). Organic acid formation closely followed the progressive consumption of fermentable sugars and was strongly associated with changes in pH and total soluble solids. Fermentation performance was further modulated by the microbial system, with mixed cultures favouring broader metabolic conversion, whereas S. thermophilus monoculture provided a more controlled and predictable biotransformation profile. The optimized fermentation strategy yielded stable fermented macroalgal pastes that maintained pH and TSS (°Brix) over 60 days of storage at room temperature. Fermentation also improved technological quality by producing softer pastes with reduced firmness and adhesiveness. Nutritionally, the fermented products retained their high mineral content, while protein content increased in G. gracilis and remained unchanged in C. tomentosum.

Furthermore, fermentation significantly enhanced the antioxidant potential of both species by increasing total phenolic content and DPPH radical-scavenging activity.

These findings demonstrate that the optimized fermentation process generated shelf-stable macroalgal food ingredients with improved technological properties, preserved nutritional quality and enhanced antioxidant functionality, highlighting fermentation as a sustainable bioprocessing strategy with strong potential for industrial food applications.

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Comparative screening of carbon sources and retail food waste streams for mycoprotein production by *Aspergillus oryzae* and *Rhizopus oligosporus var. microsporus*

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To meet the protein demand of a global population projected to hit 9.7 billion by 2050, we need to transition towards sustainable alternative protein sources, as traditional livestock production is facing critical ecological limits[1]. At the same time, food waste is a major issue. About a third of all food is lost annually, with fruit and vegetable by-products representing nearly half of this waste [2]. Transforming carbohydrate-rich food waste into high-quality fungal protein, or mycoprotein, via fermentation offers a circular economy solution to this dual challenge: mitigating food waste and satisfying protein demands. Filamentous fungi, such as Rhizopus oligosporus and Aspergillus oryzae are particularly well-suited for this purpose, as they carry a history of safe human consumption, having been utilized for centuries in traditional fermentations to produce tempeh and koji, respectively[3].

This study evaluates the capability of R. oligosporus var. microsporus and A. oryzae to metabolize simple and complex carbon sources (glucose, fructose, sucrose, cellulose, pectin, starch, galactose, and lactose). In parallel, distinct retail food waste streams (fruits, vegetables and bread) were collected and subjected to proximal characterization to map their nutritional profiles against the specific fungal growth requirements. The aim of the study is to identify the most compatible waste streams for scaling up mycoprotein production using these traditional food-grade strains.

Aerobic, small-scale submerged fermentations were conducted in shake flasks for 72 hours at 30 °C and 200 rpm. The fermentation media consisted of 10 g/L of a carbon source, supplemented with 5 g/L of peptone and 3.5 g/L of yeast extract as nitrogen sources. Flasks were inoculated with an initial spore concentration of 0.5×10^6 spores/mL of each respective fungus. Aliquots were collected at 0 h and at every 24 h to track pH and carbon source consumption. After the fermentation, the fungal biomasses were harvested to determine total biomass yield and protein content.

While A. oryzae was able to grow well on media with glucose, fructose, sucrose and galactose, with dry biomass yields ranging from 4.90 ± 0.43 to 5.57 ± 0.04 g/L, it was also able to hydrolyse pectin and starch, achieving biomass yields of 4.72 ± 0.09 and 4.66 ± 0.22 g/L, respectively. On the other hand, R. oligosporus, could not hydrolyse sucrose, pectin or starch. This fungus grew well on media with glucose, fructose and galactose, achieving dry biomass yields of 3.49 ± 0.09, 4.06 ± 0.85 and 3.51 ± 0.27 g/L, respectively. Neither of the fungi were able to hydrolyse cellulose or lactose. Across carbon sources, A. oryzae produced significantly higher overall biomass yields than R. oligosporus ($p < 0.0001$). However, R. oligosporus achieved significantly higher protein contents (37.64 ± 0.72 to 47.86 ± 0.96 %) than A. oryzae (21.58 ± 0.84 to 35.52 ± 1.03 %; $p < 0.0001$), except when grown on pectin, where no significant difference was observed.

Proximal characterization of retail food waste streams revealed distinct nutritional profiles: fruit waste was composed mainly of readily fermentable non-structural carbohydrates (64.7 %, mainly glucose and a xylose/mannose/fructose/galactose (XMFG) mix, which co-eluted under the chromatographic conditions used) with low structural carbohydrates (14.3 %, mainly glucans); vegetable waste contained 30 % non-structural carbohydrates (mainly glucose and XMFG), 17 % structural carbohydrates (mainly glucans and uronans), and 17.3 % protein. Bread waste was characterized by a high structural carbohydrate fraction (61.3 %, entirely glucans) and 9.1 % protein. Additional batch collections are ongoing to account for seasonal variability.

Integrating these compositional data with the fungal physiological profiles reveals clear targeting strategies. A. oryzae demonstrated greater metabolic versatility, making it highly suitable for directly valorising pectin- and starch-rich vegetable and bread wastes. Conversely, R. oligosporus var. microsporus is limited to simple sugars making it ideal for simple-sugar-rich fruit waste to yield high-density protein, though complex waste streams would require pre-treatment. Although actual dairy waste streams were not characterized, neither fungi were able to hydrolyse lactose, indicating that lactose-rich dairy by-products would require pre-treatments prior to fermentation. Ultimately, mapping fungal metabolism against waste composition enables strategic strain-stream pairing, balancing substrate availability with desired protein content to offer distinct pathways for targeted mycoprotein production.

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Flavors of Rome: Garum Fermentation through different techniques

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Garum, a fermented fish sauce widely consumed throughout the Roman Empire[1], was among the most important condiments of the ancient Mediterranean, rivalling olive oil in its geographical reach and culinary relevance. Traditionally produced in earthenware amphorae from fish such as anchovies, mackerel, and other species, it was frequently enriched with ingredients including wine, honey, or vinegar. Although its production largely declined after the fall of the Roman Empire, the growing popularity of fermented foods[2] has sparked renewed interest in traditional European fermented products. As a result, Garum has re-emerged as a valuable element of Europe's culinary heritage, offering both historical insight and new opportunities for the development of flavour-rich fermented foods in modern gastronomy.

Three types of Garum were studied: Garum made with Sardine (Sardina pilchardus) fermented in a food safe plastic container (Sardine 1); Garum made with Tuna (Thunnus thynnus) also fermented in a food safe plastic container (Tuna); and Garum prepared using the same Sardine recipe but fermented in a traditional earthenware container lined with a sealant made from pine resin, beeswax and olive oil (Sardine 2). All three were prepared similarly, mixing the fish with an equal weight in brine (water and crystal salt) for a final salinity of 12%, but the sardines were mixed whole while only the blood and entrails of the tuna were used. The containers were sealed in plastic film, the lids were placed and the mixtures were left to ferment in a temperature-controlled chamber at 30-40 °C for 130 days, with samples collected at the beginning of fermentation, after approximately one month and at the end of the fermentation period.

To assess the biochemical changes occurring during fermentation, samples collected at different time points were analysed for their metabolite composition. High-Performance Liquid Chromatography (HPLC) was used to monitor the production of organic acids, sugars and alcohols throughout the fermentation process. In all three garum formulations, fermentation was characterised by an increase in lactic acid and other organic acids, namely acetic, propionic and pyroglutamic acids[3]. Additionally, several sugars and alcohols, including ribose, glycerol and ethanol, accumulated over the course of fermentation.

Several differences were observed among all three samples collected after four months of fermentation. Compared with Sardine 1, the Tuna garum showed considerably higher levels of organic acids, such as malic, acetic and pyroglutamic acids, while presenting lower levels of lactic acid. The Tuna garum also showed higher concentrations of sugars and alcohols. In contrast, Sardine 2 showed lower levels of lactic and malic acid but had a sharper decrease in acetic acid. Regarding sugar and alcohol composition, both Sardine samples displayed very similar profiles.

The results confirm that different fish produce mostly the same metabolites, though tuna is far more metabolite-rich than sardine, and that using traditional fermentation amphorae can have intense effects on the acid yield of Garum. With these effects, Sardine 1 garum has a sharp, umami-intense flavour, Sardine 2 is milder and sweeter, and Tuna is sourer and tangier.

The distinct metabolic and sensory profiles obtained through different fermentation approaches demonstrate the versatility of Garum and support its reintroduction as a high-value fermented ingredient with significant potential for gastronomic innovation and market differentiation.

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Production and analysis of a craft Witbier containing whey

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The craft brewing industry has increasingly explored innovative ingredients capable of enhancing both product functionality and process sustainability. Witbier, a traditional Belgian-style wheat beer, is characterized by its pale appearance, slight turbidity, low bitterness, and distinctive fruity and spicy notes[1]. In this context, cheese whey, a major dairy industry by-product, represents a promising alternative raw material due to its nutritional composition and potential environmental benefits.

Approximately 9 L of whey are generated per kilogram of cheese produced, and its high biochemical oxygen demand (30,000–60,000 mg·L^{-1}) makes improper disposal an important environmental concern. Whey contains lactose, soluble proteins, and minerals that may contribute to beer composition and quality. In particular, whey proteins exhibit functional properties such as foaming, emulsification, and water-retention capacities, which are technologically relevant for brewing applications. However, whey addition may alter wort composition and fermentation conditions, potentially affecting yeast performance and ethanol production[2].

This study aimed to produce a Witbier-style craft beer[1] in which 25% of the brewing water was replaced with whey and to evaluate the effect of whey pH adjustment on fermentation and the physicochemical characteristics of the final beverage. Prior to use, the whey was neutralized with sodium citrate. Following brewing and fermentation, physicochemical analyses were performed[3], including moisture, total solids, ash, protein content, total soluble solids, pH, titratable acidity, and alcohol content. Ethanol concentration was determined by headspace gas chromatography after sample decarbonation. A Clarus 600 gas chromatograph, PerkinElmer, was used, with headspace sampling using an automatic sampler model CTC Analytics, Pal System. The chromatographic column was HP-INNOWAX (30 m × 0.25 mm × 0.25 µm) from Agilent.

The resulting beer presented moisture of 95.28 g/100 mL, total solids of 4.72 g/100 mL, ash content of 0.24 g/100 mL, protein content of 0.69 g/100 mL, total soluble solids of 7.2 g/100 mL, and mean pH of 4.34. The average alcohol content was 4.46% (v/v). Sensory evaluation indicated good overall acceptance of the product.

The physicochemical results demonstrate that whey incorporation significantly modified beer composition. Increased protein and soluble solids contents were attributed to the direct contribution of whey constituents, potentially enhancing body and foam related properties. The alcohol content was lower than that commonly reported for conventional Witbier formulation[1] (approximately 5.5% v/v), which may be associated with the presence of lactose, a carbohydrate not readily fermented by brewing yeast. In addition, pH, titratable acidity, and ash values suggest that minerals and organic compounds derived from both malt and whey contributed to the beverage's chemical balance.

Further analyses of volatile organic compounds, comparing Witbier formulations produced with and without whey, will be presented. Overall, partial substitution of brewing water with whey resulted in a beer with distinctive compositional and technological characteristics, highlighting the potential of whey valorization as a sustainable strategy for the development of innovative fermented beverages.

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Process monitoring and operational ranges for batch-to-batch quality control in craft beer

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Craft beer production is inherently variable, and this variability can compromise batch-to-batch reproducibility and process consistency. This study aimed to define operational ranges for key physicochemical parameters in Blonde Ale and IPA craft beers produced by a Lisbon brewery and to evaluate the effect of yeast repitching on fermentation stability. Production batches were analyzed under a two-factor design considering beer style and yeast condition (fresh vs. repitched, first reuse cycle). Fermentation and maturation were monitored through extract, pH, temperature, pressure, apparent attenuation, alcohol content, and carbonation. Data were analyzed by two-way ANOVA and Tukey's post hoc test ($p < 0.05$), and operational ranges were defined from the observed distributions and summarized as mean ± SD. Blonde Ale showed a final extract of 2.06 ± 0.35 °P, apparent attenuation of 82.9 ± 2.6%, and 5.3 ± 0.2% ABV, whereas IPA showed 3.26 ± 0.41 °P, 78.7 ± 2.8%, and 6.6 ± 0.2% ABV, reflecting differences in wort composition and fermentability. Yeast repitching significantly affected fermentation kinetics, particularly in IPA, reducing the time required to reach final extract without altering final attenuation, indicating that repitching mainly influenced fermentation rate rather than yield. pH evolution was also sensitive to yeast condition, with fresh yeast promoting deeper acidification than repitched yeast. Minimum pH values were 4.17 ± 0.08 for Blonde Ale and 4.26 ± 0.18 for IPA with fresh yeast, while repitched yeast reached 4.21 ± 0.04 and 4.41 ± 0.19, respectively. During maturation, temperature fluctuations were associated with variability in carbonation, highlighting the importance of thermal control for product consistency. Based on the observed data, operational ranges were established for Blonde Ale (final extract 1.72–2.41 °P, apparent attenuation 80.3–85.5%, and alcohol content 5.1–5.5% ABV) and IPA (final extract 2.85–3.67 °P, apparent attenuation 75.9–81.5%, and alcohol content 6.4–6.8% ABV). Complementary pH ranges were 4.09–4.25 for fresh yeast and 4.17–4.25 for repitched yeast in Blonde Ale, and 4.08–4.44 and 4.22–4.60 in IPA, respectively (Figure 1).

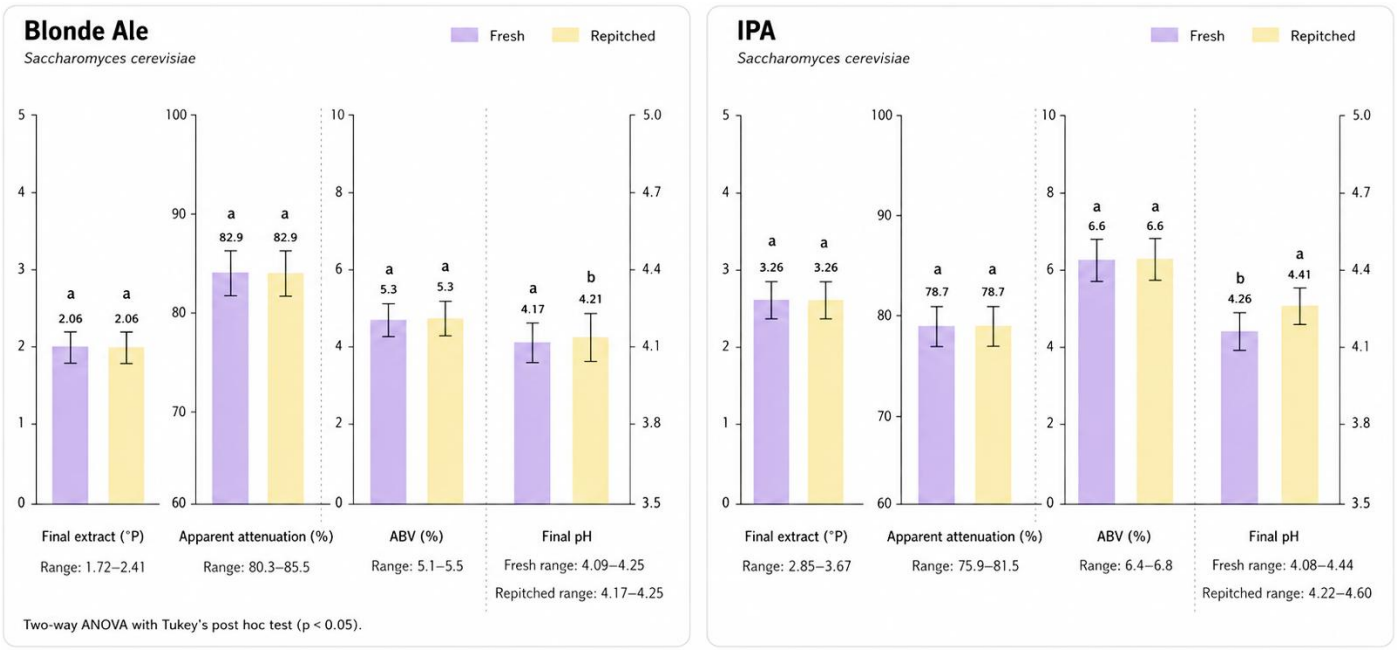


Figure 1 – Operational ranges of key physicochemical parameters in Blonde Ale (A) and IPA (B), comparing fresh and repitched Saccharomyces cerevisiae. Values are shown as mean ± SD.

These findings support the use of systematic physicochemical monitoring and statistical definition of acceptance ranges as practical tools for quality control in craft brewing.

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From tradition to precision: salt-driven microbial dynamics in Serpa PDO-inspired cheese revealed by culture-dependent and metataxonomic approaches

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The microbiological dynamics of traditional raw ewe's milk cheeses are central to their safety, quality, and sensory identity, yet remain only partially understood at the community level [1]. In the context of growing interest in sodium reduction in dairy products, this study combines classical microbiology with high-throughput 16S rRNA gene sequencing and bioinformatic analysis to evaluate how different salt concentrations shape microbial succession and community structure throughout ripening of a Serpa PDO-inspired cheese.

Cheeses were produced within the TID4AGRO project, in collaboration with Queijaria Guilherme (Serpa, Portugal), using raw ewe's milk and four salt levels (0.0%, 0.7%, 1.4% and 2.1%), the latter corresponding to the conventional Serpa PDO standard. Samples were collected at five ripening stages (days 0, 7, 15, 30 and 45), covering both typical commercial ripening and an extended maturation period.

Culture-dependent enumeration quantified total mesophilic microorganisms (PCA), lactic acid bacteria (MRS), Pseudomonas spp. (CFC) and fungi (DRBC), providing baseline hygiene and growth indicators across treatments. Total mesophilic counts were initially high, increasing further during the first weeks before stabilising, with higher salt levels associated with greater stability throughout maturation. LAB grew continuously and became dominant over time, an evolution that was more consistent in cheeses with 1.4% and 2.1% salt. Pseudomonas spp. counts rose initially but underwent a marked decline after day 7, with clear inhibition at the higher salt concentrations. Fungal populations increased up to day 7 before stabilising, with more effective control observed in higher-salt cheeses, reinforced by natamycin application.

To move beyond culturable fractions, total DNA extracted from cheese samples was used for 16S rRNA gene amplicon sequencing (V3-V4 region) on an Illumina MiSeq platform [2]. Sequence data were processed in QIIME 2, including denoising and merging with DADA2, construction of a midpoint-rooted phylogenetic tree, and calculation of alpha diversity metrics (Shannon index, Faith's phylogenetic diversity, and evenness) and beta diversity metrics (UniFrac, Bray-Curtis and Jaccard dissimilarities), visualised through principal coordinates analysis (PCoA). Statistical comparisons across salt concentrations and ripening time were performed using Kruskal-Wallis and PERMANOVA tests.

This integrated culture-dependent and metataxonomic framework allows the bacterial community structure revealed by sequencing to be directly related to the viable, technologically relevant populations quantified by classical methods, offering a more complete picture of how salt concentration modulates microbial succession, ecological stability and diversity throughout ripening than either approach alone [3]. By linking traditional cheesemaking practice to molecular ecology tools, this work contributes to a precision-oriented understanding of spontaneous fermentation processes, supporting the development of reduced-salt artisanal cheeses that preserve microbiological stability, safety and typicity.

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Lactic Acid Bacteria for Sustainable Food Fermentation: Classical vs. Molecular Identification

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Lactic acid bacteria (LAB) comprise a diverse group of Gram-positive, generally recognized as safe microorganisms with long-standing relevance in food biotechnology[1]. Their ability to acidify substrates, produce organic acids and antimicrobial compounds, contribute to aroma formation, and modulate texture makes them particularly valuable for the controlled fermentation of food raw materials and agro-industrial by-products[1,2]. Beyond preservation, LAB-driven fermentation can improve nutritional and functional quality through the reduction of anti-nutritional factors, increased bioavailability of compounds of interest, release of bioactive metabolites, and enhancement of sensory attributes[1]. These properties support their application in a wide range of matrices, including legume flours, fruit and vegetable substrates, meat and dairy products, and residues such as peels, pomace and other plant-derived fractions.

The valorization of food by-products through LAB fermentation is especially relevant in the current context of sustainable food systems and circular economy strategies. By transforming residues with low commercial value into stable, safe and potentially functional ingredients or fermented products, these microorganisms contribute to waste reduction and to the development of innovative foods with added technological and nutritional value[2]. Species belonging to the genera *Lactobacillus, Lactiplantibacillus, Lacticaseibacillus, Latilactobacillus, Levilactobacillus, Leuconostoc* and *Lactococcus* have been associated with different technological functionalities, including rapid acidification, proteolytic and carbohydrate-degrading activities, aroma development, pathogen inhibition and adaptation to specific food matrices. Representative species such as L*actobacillus acidophilus, Lactiplantibacillus plantarum, Lacticaseibacillus casei, Latilactobacillus curvatus, Levilactobacillus brevis, Lacticaseibacillus rhamnosus, Leuconostoc mesenteroides* and *Lactococcus lactis* are therefore of particular interest for strain selection and fermentation design.

Reliable identification of LAB is a critical step for their use in research and industrial applications, since technological performance, safety assessment and reproducibility are strongly dependent on strain identity. Classical identification methods, based on colony morphology, Gram staining, catalase reaction, growth conditions, carbohydrate fermentation profiles and other biochemical traits, remain useful for preliminary screening because they are accessible, inexpensive and informative at the phenotypic level. However, these approaches may lack discriminatory power when closely related species show similar metabolic profiles or when environmental conditions influence phenotypic expression. In contrast, molecular methods, such as PCR-based assays and 16S rRNA gene sequencing, provide higher taxonomic resolution, faster confirmation and greater robustness, particularly for the detection of non-cultivable or difficult-to-characterize microbial diversity. Nevertheless, molecular identification should be interpreted critically, as some closely related LAB taxa may require complementary genetic markers or combined polyphasic approaches for unequivocal discrimination[3].

In this study, some LAB strains were evaluated using both classical and molecular identification techniques, enabling a comparative assessment of their usefulness for the characterization of isolates with potential application in food fermentation. The integration of phenotypic and genotypic information strengthens the selection of strains with defined technological functionalities and supports the development of controlled, safe and reproducible fermentation processes. Overall, the results reinforce the relevance of LAB as key biological agents for innovation in food biotechnology, particularly in the transformation of food raw materials and by-products into sustainable, value-added fermented products.

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Influence of Acid Hydrolysis and Non-Thermal Plasma Cell Disruption on the Chemical and Biological Properties of Bio-Oils Produced by Rhodotorula toruloides

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The conversion of the organic fraction of municipal solid waste (OFMSW) into value-added products is a promising strategy within the circular economy. In this context, oleaginous yeasts such as *Rhodotorula toruloides* can accumulate lipids and carotenoids with potential applications in biolubricants, cosmetics, and biomaterials.[1] However, different cell disruption methods may affect the chemical composition and biological properties of the resulting bio-oils. Therefore, this study evaluated the effects of acid hydrolysis (ACHT) and non-thermal plasma (NTP) on the chemical composition, color characteristics, antioxidant capacity, antimicrobial activity, and cytotoxic potential of bio-oils produced by *R. toruloides* cultivated in OFMSW hydrolysate. Following cultivation, the biomass was subjected to cell disruption treatments, lipids were extracted using organic solvents, and the resulting bio-oils were characterized by Fourier-transform infrared spectroscopy (FTIR), CIELAB colorimetry, total phenolic content (TPC), ABTS radical scavenging assay, disk diffusion antimicrobial assays, and cytotoxicity evaluation using MDA-MB-231 cells.

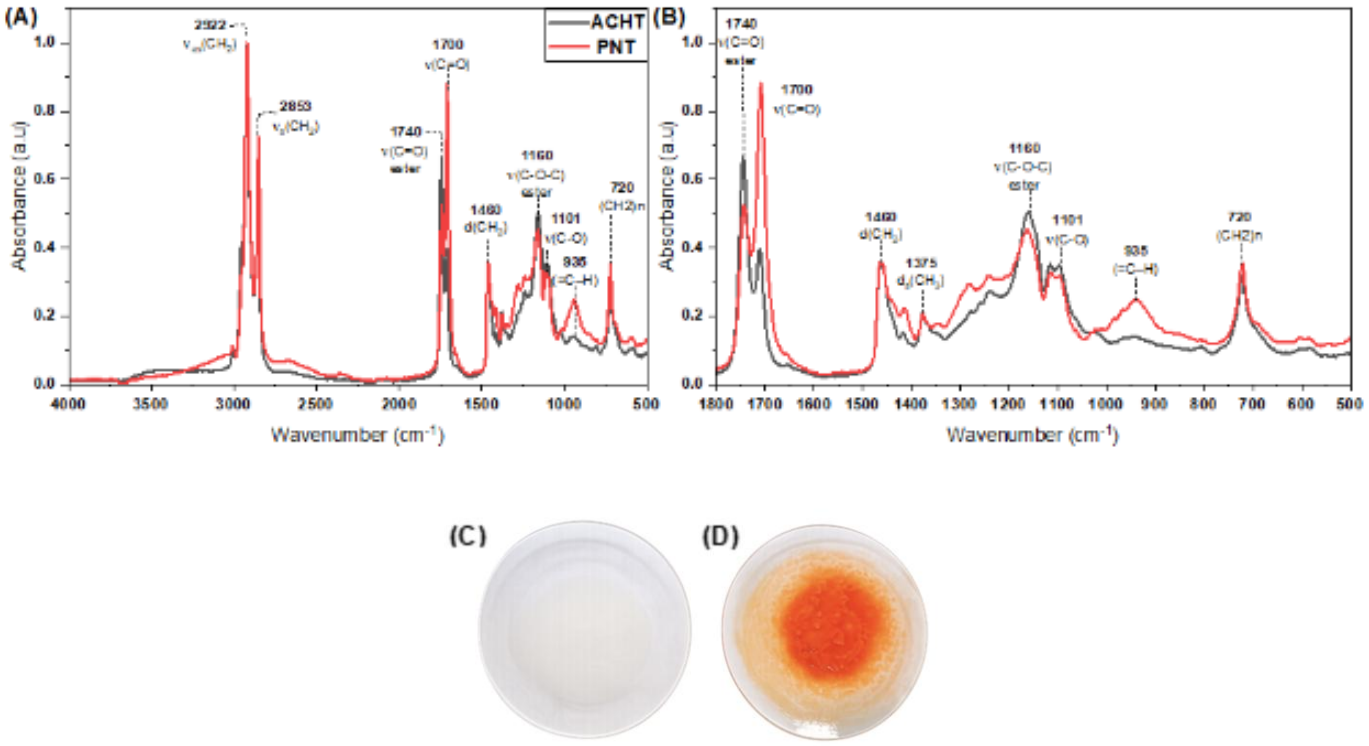


Figure 1 - FTIR spectra of bio-oils obtained from *R. toruloides* subjected to ACHT and NTP. (A) Full spectrum from 4000 to 500 cm^{-1}. (B) Fingerprint region from 1800 to 500 cm^{-1}. (C) Image of sample PNT. (D) Image of sample ACHT.

Chemical characterization revealed typical lipid-associated bands in both bio-oils. In the full FTIR spectrum (Figure 1A), the bands at 2922 and 2853 cm^{-1} were assigned to the asymmetric and symmetric stretching vibrations of CH_2 groups present in fatty acid and triacylglycerol aliphatic chains, whereas bands observed at 1740 and 1700 cm^{-1} corresponded to carbonyl stretching of lipid esters and free fatty acids or oxygenated carbonyl compounds, respectively.[2] Compared with ACHT, the NTP bio-oil exhibited a higher relative intensity in these regions, indicating differences in the recovered lipid fractions. In the fingerprint region (Figure 1B), bands at 1460 and 1375 cm^{-1} were attributed to CH_2 and CH_3 deformations, bands at 1160 and 1101 cm^{-1} to C–O–C and C–O stretching of lipid esters, and the band at 720 cm^{-1} to rocking vibrations of $(CH_2)n$ groups.[2] Overall, FTIR demonstrated that the cell disruption method influenced the chemical composition of the bio-oils. The visual

appearance of the bio-oils (Figure 1C–D) was consistent with the colorimetric analysis, revealing marked differences between the cell disruption methods. The ACHT bio-oil exhibited an intense orange coloration, whereas the NTP bio-oil appeared considerably lighter. These observations were confirmed by the CIELAB parameters, with ACHT showing lower lightness (L = 31.19 ± 0.06), higher *a** (24.63 ± 0.42) and b* (39.39 ± 4.58) values, while NTP presented higher lightness (*L** = 60.83 ± 0.32), negative *a** (-3.71 ± 0.15) and lower *b** (15.34 ± 0.19). The distinct color profiles suggest differences in the relative contribution of pigmented compounds, including carotenoids produced by *Rhodotorula*.[1] TPC was higher in ACHT (544.01 ± 65.40 mg GAE/g oil) than in NTP (460.32 ± 19.32 mg GAE/g oil). Likewise, ACHT exhibited greater ABTS radical scavenging activity (23.89 ± 6.27%) than NTP (5.44 ± 1.94%), corresponding to 0.121 ± 0.030 and 0.014 ± 0.007 mg TE/g oil, respectively, indicating that the cell disruption method influenced the antioxidant-related properties of the bio-oils. The antibacterial activity was assessed using the agar well diffusion method by measuring the diameter of the inhibition zones against Gram-positive bacteria (*Staphylococcus aureus, Listeria monocytogenes, and Bacillus cereus*) and the Gram-negative bacterium (*Escherichia coli*). Antibacterial activity was detected only for NTP, which produced inhibition zones against all tested bacteria, whereas ACHT showed no detectable antimicrobial activity. Cytotoxicity assays revealed IC_{50} values of 303.38 ± 23.68 and 237.04 ± 12.04 µL/mL for ACHT after 24 and 48 h, respectively, while NTP showed lower values (262.07 ± 4.27 and 212.01 ± 4.86 µL/mL), indicating greater cytotoxic activity. Together with the FTIR results, these findings suggest that changes in the chemical composition of the NTP bio-oil were accompanied by enhanced biological activity.[1,3] Overall, the cell disruption strategy significantly influenced the chemical composition and functional properties of *R. toruloides* bio-oils. Acid hydrolysis yielded bio-oils with higher antioxidant-related properties, whereas non-thermal plasma produced distinct FTIR profiles and enhanced antimicrobial and cytotoxic activities. These findings highlight cell disruption as a key processing step for tailoring microbial bio-oils obtained from OFMSW for value-added applications within the circular bioeconomy.

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Innovative Artisanal Cider: Fermentation Monitoring and Functional Potential of a Novel Fruit Juice Blend

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The incorporation of functional ingredients, such as microalgae, into traditional fermented beverages represents a promising strategy for the developing of innovative products with added nutritional value. Thus, such formulations require strict control of the fermentation parameters to ensure the process efficiency, product quality and batch-to-batch consistency. In this context, this study evaluated the fermentation and final characteristics of an artisanal cider enriched with a fruit juice blend with spirulina (**Figure 1**), assessing the effect of initial total soluble solids (TSS) content, density, fermentation time and final alcohol content. The fermentation was carried out using 2.25 L of apple juice and 750 mL of a mixture of apple juice (65%), coconut water (13%), grape juice (11%), coconut water (13%), guava purée (5%), passion fruit juice (3%), lime juice and spirulina concentrate (3%) commercially available in order to improve nutritional and functional properties of the substrate. To enhance the fermentable sugar content, the initial TSS was raised to 13 ºBrix through sucrose addition, yielding the initial density to 1.052 g/mL. The mixture was inoculated with 5 g of rehydrated dry yeast (Saf CiderTM TF-6), corresponding to a concentration of approximately 1.67 g/L, and fermented under anaerobic conditions at approximately 18.5ºC. Following the primary fermentation, the cider was stabilized and subjected to bottle conditioning with the addition of 9 g/L of sucrose to the mixture. The incorporation of the fruit juice blend with spirulina did not compromise the fermentation performance, with consistent and efficient sugar conversion into alcohol observed throughout the process. The final cider (clarified) resulted in 2L with a final density decreased to 1.000 g/mL with a 5 ºBrix and an alcohol content of approximately 6% (v/v). The results confirm that the incorporation of fruit juice blends with spirulina can be successfully incorporated into cider formulations without negatively affecting fermentation kinetics of alcohol content. This work supports the potential of microalgae-enriched fermented beverages as a novel approach for the valorization of functional ingredients in artisanal cider production.

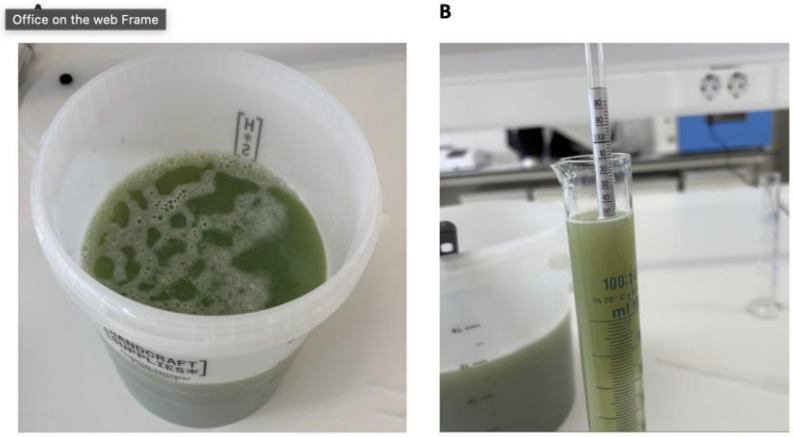


Figure 1 – Initial stage of fermentation (A) and Measurement of initial density (B).

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Solid-state fermentation of Chickpea for the development of functional protein ingredients

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Worldwide nutritional demands are increasingly shifting toward healthier and more sustainable food systems, with a particular emphasis on improving dietary protein availability. In this context, alternative (i.e., non-animal) protein sources for human consumption, such as plant-based and insect-based proteins have been gaining attention. Notwithstanding their availability and potential as protein source for human consumption, these alternative proteins often exhibit limitation in sensory quality, techno-functional properties, and digestibility. To overcome these shortcomings of alternative proteins, various advanced processing technologies (e.g., Ohmic Heating, High-Pressure Processing, Supercritical Fluid Extraction, etc.) have been explored to improve their extraction, tecno-functional properties and digestibility. Alternatively, to these methodologies, fermentation, a millennial processing methodology, has been re-gaining popularity to enhance the quality of alternative proteins. Through microbial activity, fermentation modifies the food matrix, contributing to improved texture, flavor, and nutrient accessibility.

This study aimed at improving the nutritional and functional properties of proteins from legumes through solid-state fermentation. Chickpea (Cicer arietinum L.) was fermented using Rhizopus oligosporus to obtain chickpea Tempeh. The methodology employed consisted in soaking the grains for 18h with water followed by their pre-processing, cooling and inoculation in perforated stomacher bags (of food-grade low-density polyethylene (LDPE)) and incubation for 40 h at 30 °C. Different samples were obtained by varying grain pre-processing methodology including: boiling for 1 h; autoclaving for 25 min at 121 °C; dehulling and boiling for 1 h; dehulling and autoclaving for 25 min at 121 °C; grinding and boiling for 1 h; grinding and autoclaving for 25 min at 121 °C. The resulting samples were stabilized (by atmospheric steaming for 30 min), lyophilized, and milled into tempeh flour. A non-fermented control was also prepared.

Fermentation yielded cohesive products with characteristic white mycelium and a nutty aroma. Protein content (determined by DUMAs) ranged from 16.69 ± 0.57% in the control to up to 20.61 ± 0.39% in fermented samples, with autoclaved and dehulled treatments showing significant increases. Reducing sugars increased significantly in all fermented samples (up to 2.68 ± 0.29%), indicating partial carbohydrate hydrolysis. Water holding capacity improved markedly, showing a 2–3-fold increase compared to the control.

Overall, solid-state fermentation with Rhizopus oligosporus effectively enhanced both the nutritional and functional attributes of chickpea. The observed increase in protein content, combined with higher levels of reducing sugars and improved water holding capacity, reflects structural modifications of the matrix driven by fungal metabolism. These changes suggest improved protein availability and potential enhancement of digestibility. The results support fermentation as a robust, low-tech strategy to valorize legumes into higher-value, protein-rich ingredients with improved techno-functional performance.

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Evaluation of Phenolics Fate, Bioaccessibility, and Intestinal Permeability in Coffee By-product Kombuchas: Impact of Fermentation Time

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Kombucha is a popular functional drink typically crafted from Camellia sinensis. Utilizing coffee by-products, including leaves, cascara, and roasted beans, presents a sustainable alternative due to their high phenolic content1. Nevertheless, how these bioactive molecules evolve during extended fermentation, along with their subsequent digestive stability and intestinal absorption, remains poorly understood. This study aimed to investigate how fermentation duration influences the stability, bioaccessibility, and intestinal epithelial transport of caffeine and fifteen phenolic compounds in innovative coffee-derived kombuchas.

Infusions prepared from roasted coffee beans, leaves, and cascara (3% w/v, steeped at 95 °C for 10 min) were fermented with 10% sugar, 2.5% SCOBY, and a specialized Kombucha Consortium (pre-adapted through prior cultivation in green and black tea) at 23 °C to produce kombuchas. Samples were collected on days 0, 6, and 9 2,3. Bioaccessibility was modeled using the standardized static in vitro INFOGEST 2.0 digestion framework. Intestinal permeability was then assessed over a 180-minute window using a Caco-2/HT-29 cell co-culture monolayer. Quantification of caffeine and distinct phenolic compounds across fermentation stages, post-digestion phases, and apical/basolateral compartments was performed via HPLC-DAD and LC-MS.

Chromatographic profiling revealed that day 6 (T6) represented the optimal fermentation window for retaining bioactive constituents, whereas extending the process to day 9 (T9) triggered extensive degradation (Figure 1A).

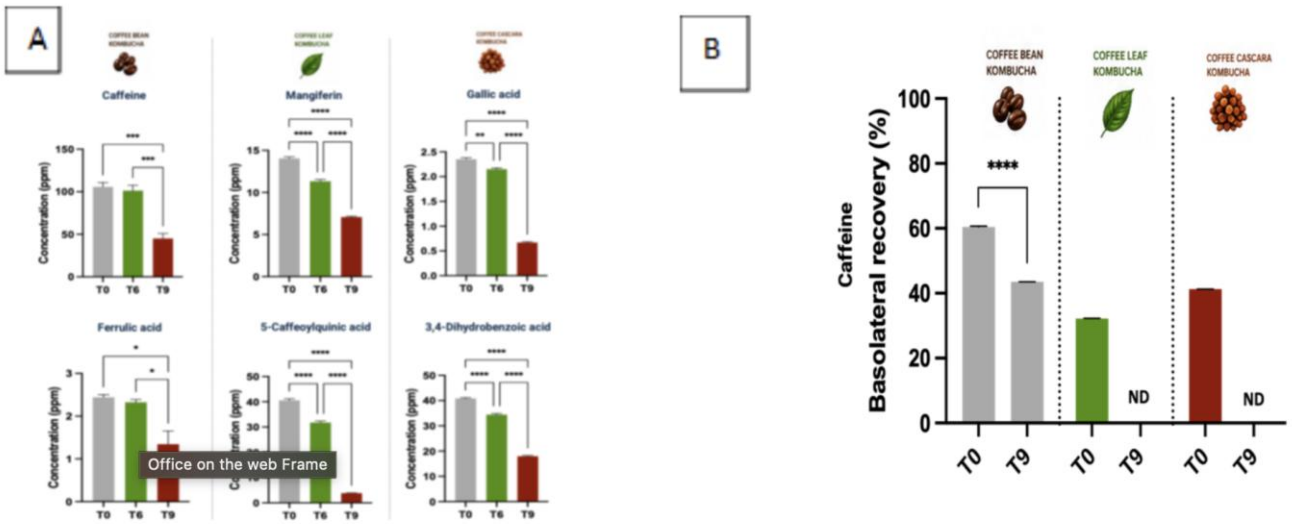


Figure 1 - (A) Representative compounds illustrating fermentation-dependent degradation pattern across kombucha matrices. *** p<0.001; **** p<0.0001. (B) Basolateral recovery (%) of caffeine in different coffee-derived kombuchas across time. **** p<0.0001; ND, not detected

Parallel degradation patterns occurred in cascara and leaf matrices, notably impacting mangiferin and 5-caffeoylquinic acid. Following simulated gastrointestinal digestion, T6 variants yielded significantly higher concentrations of bioaccessible phenolics compared to T9, with leaf infusions experiencing the most severe drop (~60%). Cell transport assays highlighted that intact parent phenolics possessed low permeability across the intestinal barrier, with only trace levels of low-molecular-weight metabolites (e.g., ferulic and 3,4-dihydroxybenzoic acids) reaching the basolateral side, while caffeine is readily transported but decreased after prolonged fermentation (Figure 1B). Prolonging kombucha fermentation to 9 days negatively impacts phytochemical integrity and subsequent bioaccessible yields in a matrix-specific manner. These findings identify fermentation time as a critical parameter shaping the stability, bioaccessibility and potential intestinal availability of bioactive compounds in coffee-derived kombuchas.

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Development of a Portuguese Kimchi using starter cultures and local Ingredients

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Kimchi is a traditional Korean fermented food produced through the lactic acid fermentation of vegetables, primarily Chinese cabbage, combined with garlic, ginger, chilli pepper and other seasonings. The fermentation process, driven by lactic acid bacteria, promotes pH reduction, enhances microbiological safety, and contributes to the sensory and nutritional characteristics of the product[1].

In this study, an innovative Portuguese-style kimchi was developed based on the study from Korus et al. (2021) by replacing Chinese cabbage with Portuguese heart cabbage (Brassica oleracea) and incorporating traditional national ingredients such as coriander, bay leaves and paprika. To improve fermentation control and reproducibility, a starter culture of Lactiplantibacillus plantarum and Leuconostoc mesenteroides was employed, representing a precision fermentation approach applied to a traditional fermented food[2].

Process optimisation was performed by evaluating four experimental conditions (in duplicate) combining the presence or absence of broken-rice paste (WR, WOR) and a hot pepper by-product (WP, WOP), thereby contributing to a circular economy strategy. The fermentation was performed at 4 °C for up to 28 days, during which the following physicochemical parameters were monitored periodically: pH, titratable acidity (AOAC 942.15), total soluble solids (°Brix), sugars quantification and organic acids by HPLC.

The formulation containing both pepper by-product and broken-rice paste (CH/CP) exhibited the most favourable fermentation profile, showing greater acidification (pH ~4, titratable acidity 1.26 g lactic acid/100 g fresh kimchi) and more efficient substrate utilisation, evidenced by the reduction of glucose, xylose and maltose to 8.3, 3.9 and 1.1 g/mL, respectively, after 28 days of fermentation.

This formulation was therefore selected for nutritional characterization – protein, fat, carbohydrates, dietary fibre, salt - determined by combustion method (DUMAS), AOAC 922.06, AOAC 991.43, AOAC 935.47 methods, respectively. Carbohydrates and energy value were calculated by difference using the moisture value also assessed. The selected kimchi formulation presented 2.59 % protein, 0.18 % lipids, 0.34 % total dietary fibre, 2.28 % carbohydrates, 1.32 % salt (% per 100g of fresh biomass), and an energy value of 28.5 kcal/100g.

In addition, fermentation significantly improved the antioxidant capacity of the selected formulation, increasing DPPH radical-scavenging capacity from 13.47 to 17.35 mmol TEAC/g (+ 28.8 %) and ABTS activity from 4.25 to 24.34 mmol TEAC/g (+ 472.7 %), demonstrating that the fermentation process significantly enhanced the bioactive potential of the kimchi. These results demonstrate that the developed kimchi is a low-fat, nutritionally valuable and safe alternative within the fermented food category.

Overall, the findings demonstrate that adapting kimchi production to Portuguese raw materials, combined with the use of defined starter cultures, enabled the development of an innovative fermented food with enhanced nutritional value and the potential to meet the growing consumer demand for functional fermented products produced from local ingredients.

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Sustainable Recovery of Hydroxytyrosol for Food and Nutraceutical Applications: An Integrated Solvent Screening Approach

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Hydroxytyrosol (HT) is a phenolic alcohol predominantly found in olive-derived extracts and is a high-value compound of industrial interest, particularly in the food and nutraceutical sectors, due to its antioxidant, anti-inflammatory, and neuroprotective properties, which have been associated with the prevention of several diseases, including diabetes, neurodegenerative disorders, and cancer[1,2]. However, HT is present in complex natural matrices, and its large-scale production remains challenging, highlighting the need to develop more efficient downstream processing strategies[3]. In this study, a computational pre-screening approach based on the COSMO-RS was applied to predict the interactions between 32 solvents and hydroxytyrosol, enabling rapid and cost-effective solvent screening prior to laboratory experiments. Subsequently, these solvents were evaluated through experimental screening using liquid-liquid extraction (LLE) at a 1:1 (v/v) phase ratio. Extraction performance was assessed based on the partition coefficient (K) and extraction efficiency (EE), with both phases analyzed by ultra-high-performance liquid chromatography (UHPLC). Interaction coefficient values ranging from −2.271 to 12.311 were predicted by COSMO-RS, with more negative values indicating a higher affinity for HT. Experimentally, the best-performing solvents achieved K values ranging from 1.15 to 3.75 and EE values between 55% and 86%. Overall, this study demonstrates that COSMO-RS is an effective tool for preliminary solvent selection, supporting the rational identification of promising solvents and contributing to the development of more efficient and sustainable extraction processes for hydroxytyrosol.

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Enzymatic Hydrolysis of Sugar Beet Molasses as an Alternative Glucose Source for Microalgae Cultivation

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Sugar beet molasses is an abundant agro-industrial by-product generated during sugar beet processing and represents a promising sustainable feedstock for the production of fermentable sugars. Rich in sucrose and other carbon-containing compounds, molasses can serve as a low-cost carbon source for heterotrophic microalgae cultivation, thereby reducing the environmental footprint associated with conventional culture media.

This study evaluated the enzymatic hydrolysis of sugar beet molasses to optimize reducing sugar production while minimizing enzyme consumption and processing costs. In addition, the resulting hydrolysate was evaluated as a partial substitute for pure glucose in the cultivation of *Chlorella* spp. The effects of enzyme concentration (30–300 U/g), incubation time (1–6 h), and substrate pre-treatment (dilution with water, pH adjustment to 5.0, and acid treatment with 5 M H_2SO_4) on reducing sugar yield were investigated. Molasses was hydrolysed using invertase (Invertase from baker's yeast, Grade VII, Sigma I4504) at 55 °C and reducing sugars quantified using the 3,5-dinitrosalicylic acid (DNS) method. The hydrolysate was subsequently tested as a partial replacement for pure glucose in heterotrophic culture media for *Chlorella vulgaris* WT and *Chlorella sorokiniana* WT. Microalgae were cultivated under standard conditions (28°C, 125 rpm, in the dark) in media containing a total carbon concentration equivalent to 20 g/L glucose, with glucose replaced by hydrolysate at levels ranging from 0 to 100%. Response surface methodology (RSM) was used to evaluate factor interactions and identify optimal hydrolysis conditions, while statistical significance was assessed by ANOVA ($p < 0.05$).

Substrate pre-treatment had a greater influence on hydrolysis efficiency than enzyme concentration ($p < 0.05$). The highest reducing sugar yield (738.6 g/L) was obtained from diluted acid-treated molasses, hydrolysed with 30 U/g invertase after 6 h of incubation. The absence of pre-treatment with acid also produced high sugar concentrations (629.5 g/L), although requiring a higher invertase concentration (150 U/g). Increasing enzyme concentration resulted in only marginal improvements, indicating enzyme saturation. Response surface analysis confirmed that enzyme concentrations ranging 30 – 150 U/g and incubation time of approximately 2-3 h were sufficient to achieve near-maximal hydrolysis under most conditions. In contrast, desugared molasses, a by-product of secondary sugar recovery, consistently produced significantly lower reducing sugar concentrations (<280 g/L) due to its reduced sucrose content. When incorporated into Chlorella culture media, biomass production decreased ($p < 0.05$) when glucose was replaced by 80% or 100% molasses hydrolysate. However, no significant differences ($p > 0.05$) compared to control were observed when glucose was replaced by 20–60% hydrolysate.

Invertase proved highly effective in hydrolysing the sucrose present in sugar beet molasses into the reducing sugars. When both sugar yield and enzyme cost were evaluated, the most economically viable approach was found to be offered by a low enzyme load (30 U/g) when used in conjunction with an optimised pre-treatment. Based on these findings, it is concluded that the tailoring of the pre-treatment step is paramount to the maximisation of sugar extraction. Furthermore, up to 60% of pure glucose in the heterotrophic culture of *Chlorella* spp. can be replaced by this hydrolysate, with no adverse effects being observed on biomass accumulation or cellular health. Overall, these results position sugar beet molasses as a promising, sustainable carbon source, facilitating the upcycling of industrial residues and reinforcing the principles of the circular bioeconomy across microalgae cultivation.

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From Apple Pulp to Brain Health: Valorizing Dietary Fibers for Psychobiotic Modulation

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The microbiota-gut-brain axis plays a fundamental role in mental health through the production of neuroactive metabolites by specific microorganisms known as psychobiotics.[1] This project aims to investigate the potential of fruit pulps, an undervalued co-product of the juice industry, as sustainable sources of soluble dietary fibres to selectively promote these beneficial bacterial populations.[2] Apple pulp was selected as a model fruit matrix and characterized for its moisture, dietary fibre and carbohydrate composition. Moisture content was 77%, while insoluble dietary fibre (IDF, 4.8%) and soluble dietary fibre (SDF, 2.0%) were determined using the Megazyme K-RINTDF enzymatic kit. The IDF fraction comprised 84.1% carbohydrates, mainly cellulose (54 mol% glucose), together with pectic polysaccharides (8 mol% galacturonic acid, 10 mol% galactose and 11 mol% arabinose) and xyloglucans (11 mol% xylose). The SDF fraction contained 62.4% carbohydrates and was predominantly composed of pectic polysaccharides (47 mol% galacturonic acid, 14 mol% galactose and 29 mol% arabinose). Monosaccharide composition was determined by sugar analysis following derivatization into alditol acetates and analysis through gas chromatography with flame ionization detection (GC-FID), while free sugars (0.5% glucitol, 3% glucose, 7% fructose and 2% sucrose) were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

To overcome the limited solubility of the insoluble fibre fraction, enzymatic hydrolysis using the commercial food-grade enzymes Celluclast and Fructozym will be performed to selectively depolymerize cellulosic and pectic polysaccharides, enhancing fibre solubility and generating oligosaccharides. These products will be fractionated by size-exclusion chromatography and ultrafiltration and further characterized through glycosidic linkage analysis, with the oligosaccharides analysed by high-performance anion-exchange chromatography and electrospray ionization mass spectrometry.

Subsequently, the fibre-rich fractions will be subjected to in vitro digestion and fermentation using human faecal inocula.[3] This approach will enable the analysis of oligosaccharide consumption and the quantification of short-chain fatty acids and neuroactive metabolites, including gamma-aminobutyric acid (GABA) and tryptophan derivatives,[1] produced during fermentation, alongside microbiota profiling by 16S rRNA gene sequencing. The results will establish correlations between specific carbohydrate structures and the selective growth of bacterial genera associated with mental health, such as *Bifidobacterium* and *Lactobacillus*.[1,2] Furthermore, fermentation-derived metabolites will be applied to neuronal cell cultures to evaluate their effects on synaptic connectivity and neurotrophic responses. By integrating detailed carbohydrate characterization with structure-function analysis, this project aims to demonstrate how the valorization of fruit co-products through targeted enzymatic fibre modulation can promote psychobiotic populations, providing a sustainable strategy for the development of prebiotic ingredients to support brain health.

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Cheese Whey as an Effective Fermentation Medium for Bacteriocin Production against Listeria monocytogenes

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Fermentation by lactic acid bacteria (LAB) has been used for centuries to preserve food and make it more palatable. These microorganisms may also generate bioactive compounds such as bacteriocins — ribosomal synthesized antimicrobial peptides that are effective at low concentrations, heat-stable, active across a broad pH range, and generally non-toxic. Yet, despite these advantages, commercial bacteriocin application remains constrained by high production costs and low purification yields. A sustainable solution lies in using whey — a nutrient-rich by-product of cheese production — as a food-grade fermentation substrate to produce bacteriocins. Previous studies have isolated a bacteriocin producer from Azorean artisanal cheeses, Enterococcus faecalis L3A21K6, which exhibits activity against the pathogen Listeria monocytogenes.

The aim of this study was to determine whether cheese whey could serve as a fermentation substrate to support the growth of this strain and the production of bacteriocins. Both acid and sweet whey were tested, and fermentation parameters (pH and time) were optimized to maximize yield. An overnight culture of E. faecalis L3A21K6 (grown in MRS broth at 30 °C) was centrifuged (4000 × g, 20 min), and the resulting pellet was washed with phosphate-buffered saline (PBS). The washed pellet was then resuspended in pasteurized cheese whey (65 °C for 30 min) and incubated at 30 °C with hourly sampling. Viable cell counts were determined on MRS agar after 48–72 h of incubation at 30 °C. For determination of the bacteriocin minimum inhibitory concentration (MIC), samples were centrifuged at 4000 × g for 10 min, and the supernatant was heated to 100 °C for 5 min to inactivate proteases. The supernatant was then neutralized with phosphate buffer (pH 7.0) and filter-sterilized (0.22 µm, Sartorius, Germany) to obtain the cell-free supernatant (CFS). For the MIC assay, each CFS was serially diluted twofold with sterile PBS, and bacteriocin activity against L. monocytogenes ATCC 7466 was assessed using a well-diffusion assay (WDA).

Bacterial growth at 30 °C was observed in both sweet whey and acid whey (initial pH 5–6), with the strain consistently reaching stationary phase within 24 h of incubation. Throughout fermentation, the pH decreased by 1.9 and 0.98 units in sweet and acid whey, respectively. Bacteriocin production was strongly influenced by the substrate composition and initial pH. In sweet whey, maximum activity reached 533 AU/mL between 24 and 48 h, with a slight reduction observed after 72 h. Notably, acid whey (pH 5–6) supported significantly higher bacteriocin yields, achieving a peak activity of 2133 AU/mL at 72 h, which was fourfold greater than in sweet whey. In contrast, growth in strongly acidified whey (pH 4.6) was impaired; bacterial counts declined progressively by approximately 1 log unit over 72 h, and no bacteriocin activity was detected. However, neutralizing this highly acidic whey to pH 6.5 with NaOH fully restored bacteriocinogenic capacity. Under these neutralized conditions, activity was first detected after 6 h of incubation, corresponding to the mid-logarithmic phase (approx. 10^7 CFU/mL). Maximum activity (1067 AU/mL) was reached at 24 h, coinciding with the transition from late-logarithmic to early stationary phase, after which activity gradually declined with extended incubation.

In conclusion, these findings establish the initial pH of whey as a critical determinant of both strain viability and bacteriocin production. While strongly acidic conditions (pH 4.6) are detrimental to cell survival and completely suppress bacteriocin synthesis, neutralization effectively rescues production, underscoring the importance of maintaining a permissive pH threshold. More importantly, acid whey at pH 5–6 proved to be a superior substrate to sweet whey, yielding considerably higher bacteriocin titers over an extended fermentation period. This suggests that acid whey—a major dairy by-product—can be valorized as a highly efficient and cost-effective production medium, if pH is adequately managed to support robust cellular metabolism and sustained bacteriocin synthesis.

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XVIII Encontro de Química dos Alimentos

Coimbra | 14 - 16 de outubro'26

SISTEMAS ALIMENTARES, SEGURANÇA, AUTENTICIDADE, RASTREABILIDADE, IMPACTO SOCIAL E INTELIGÊNCIA ARTIFICIAL



Investigadora Coordenadora do Laboratório Nacional de Engenharia Civil (LNEC), onde dirige o Núcleo de Engenharia Sanitária. Engenheira Química e doutorada em Engenharia Química, é especialista nas áreas da qualidade, tratamento e reutilização de água. Foi professora universitária entre 1997 e 2006 e tem desempenhado funções em diversas organizações nacionais e internacionais ligadas à gestão e investigação em recursos hídricos. É mentora do grupo especialista em Água e Clima da *Water Europe*, membro do Comité de Gestão do Grupo Especialista em Reutilização de Água da *International Water Association* e membro fundador do comité técnico da ISO dedicado à reutilização de água. Tem liderado projetos nacionais e europeus nas áreas da qualidade e tratamento da água, avaliação de risco, gestão de ativos, regulação e desenvolvimento de políticas públicas.

The role of water in food security and safety

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Water plays a key role in food security and safety. It is a food in itself and an essential factor in food production and processing, irrespective of whether diets are animal- or plant-based. Water is intrinsically linked to other key elements of food production (F), namely energy (E) and nutrients, particularly phosphorus (P), as expressed by the W-E-P-F nexus. It can also be a route of exposure to microbiological, chemical and physical hazards. Threats to water availability, in both quantity and quality, are therefore threats to food security and safety. This communication discusses major challenges (Figures 1 and 2) and solutions (examples in Figure 3) in a context of increasing water scarcity and deterioration of water-body quality, driven by climate change and water overexploitation for agricultural, industrial, urban and energy-production purposes.

Water efficiency and water reuse are central measures of the European Water Resilience Strategy (COM/2025/280 final) and are particularly relevant to the resilience of food systems and the transition towards circular-economy models[1]. Ensuring water availability, quality and sustainable use simultaneously is a major challenge for water and food security and safety. Water reuse should therefore move beyond being considered solely a response to scarcity and become part of a water-management strategy based on fit-for-purpose quality and protection of public health and the environment (Figure 2). This approach is particularly important where increasing competition among water uses requires allocation decisions that account not only for resource availability but also for the quality required by each application[1].

Emerging contaminants, including pharmaceuticals, PFAS, microplastics, and antibiotic-resistant microorganisms and genes, reinforce the need for robust monitoring, adequate treatment and continuous risk assessment[2]. Integrating these tools with food-safety management systems such as HACCP and ISO 22000, and with One Health approaches, enables an integrated consideration of water, food, environment and health. Different water uses require different quality standards, treatment levels, monitoring strategies and risk-management approaches, supported by specific legislation.

EU and national legislation have evolved to promote water efficiency and safety for different uses by updating quality requirements to address emerging hazards and adopting risk assessment and management approach from source to tap/use. The Drinking Water Directive and its Portuguese implementation, including Decree-Law 149/2026 and ERSAR Regulation 976/2025 on products and materials in contact with water, establish drinking-water quality requirements, risk assessment and water-loss control. Regulation (EU) 2020/741 establishes minimum quality, monitoring and risk-management requirements for water reuse in agricultural irrigation, while Decree-Law 119/2019 regulates reclaimed-water uses for non-potable agricultural, industrial and urban applications in Portugal. Directive (EU) 2024/3019 promotes enhanced urban wastewater treatment, including stricter nutrient requirements, micropollutant removal, AMR monitoring, energy neutrality, water reuse and resource recovery, notably phosphorus, a critical nutrient for food production and a major driver of eutrophication. This evolution links pollution control with circularity by recovering water, energy and nutrients while reducing pressures on receiving waters and primary resources.

ERSAR annually assesses and benchmarks water-service quality in mainland Portugal through performance indicators. Its fourth-generation system anticipates recent legal developments by updating the “Safe Water” indicator and testing a water-reuse indicator that accounts for urbanisation and regional water scarcity, promoting reuse where scarcity and proximity between treatment plants and water-use sites make it most relevant. The indicator is thus intended to reflect territorial context rather than applying a single reuse target to all utilities.

Under extreme water-scarcity conditions, potable reuse may also be considered (not yet in Portugal), either indirect (IPR) or direct (DPR)[3]. The feasibility of DPR was recently demonstrated at pilot scale for a food-industry application, using advanced wastewater treatment to produce water for beer production[3].

Overall, safe matching of water quantity and quality to the final use, supported by smart water allocation (fit-for-purpose water quality) and sound risk management, including monitoring (Figure 3), is essential to reconcile water-resource efficiency, health and environmental protection, equitable access to safe water, and food security and safety.

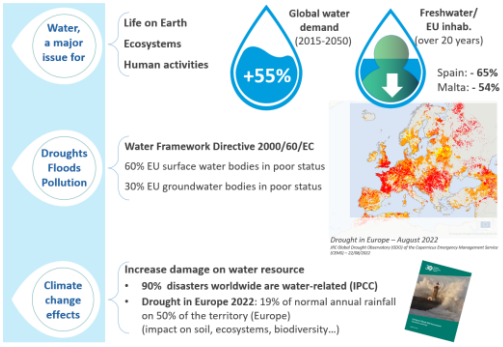


Figure 1 – Water challenges for ensuring water security for all, addressed by the Water4All partnership.

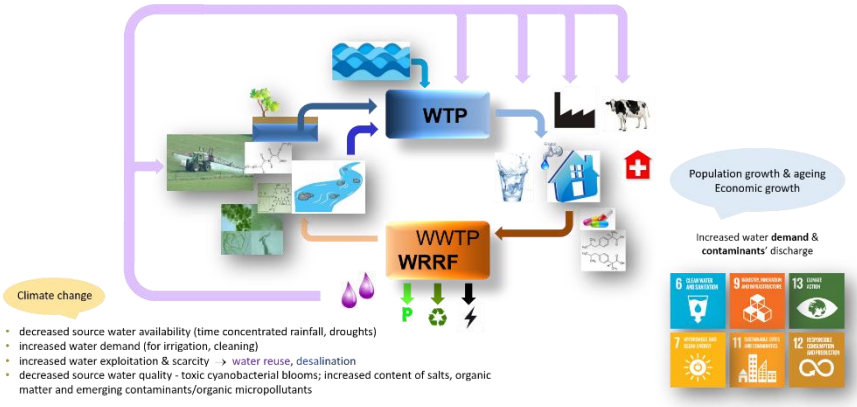


Figure 2 – Water quantity and quality challenges and urban-agriculture-industrial symbiosis.

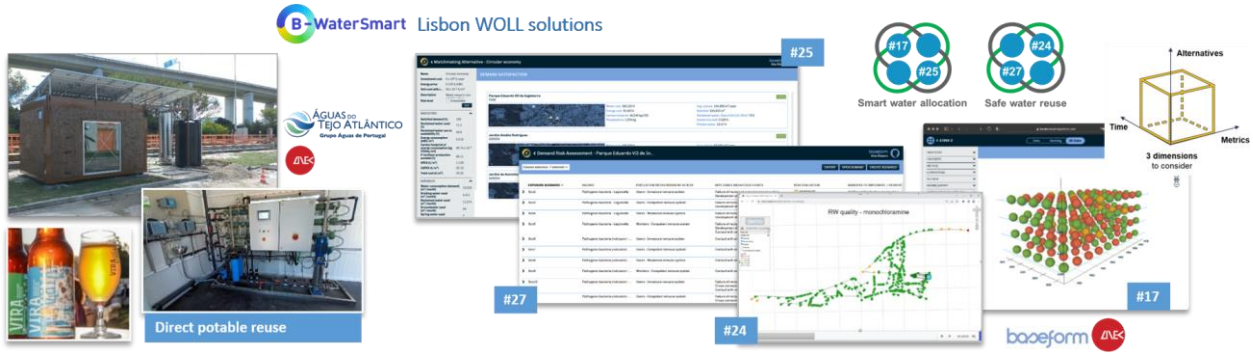
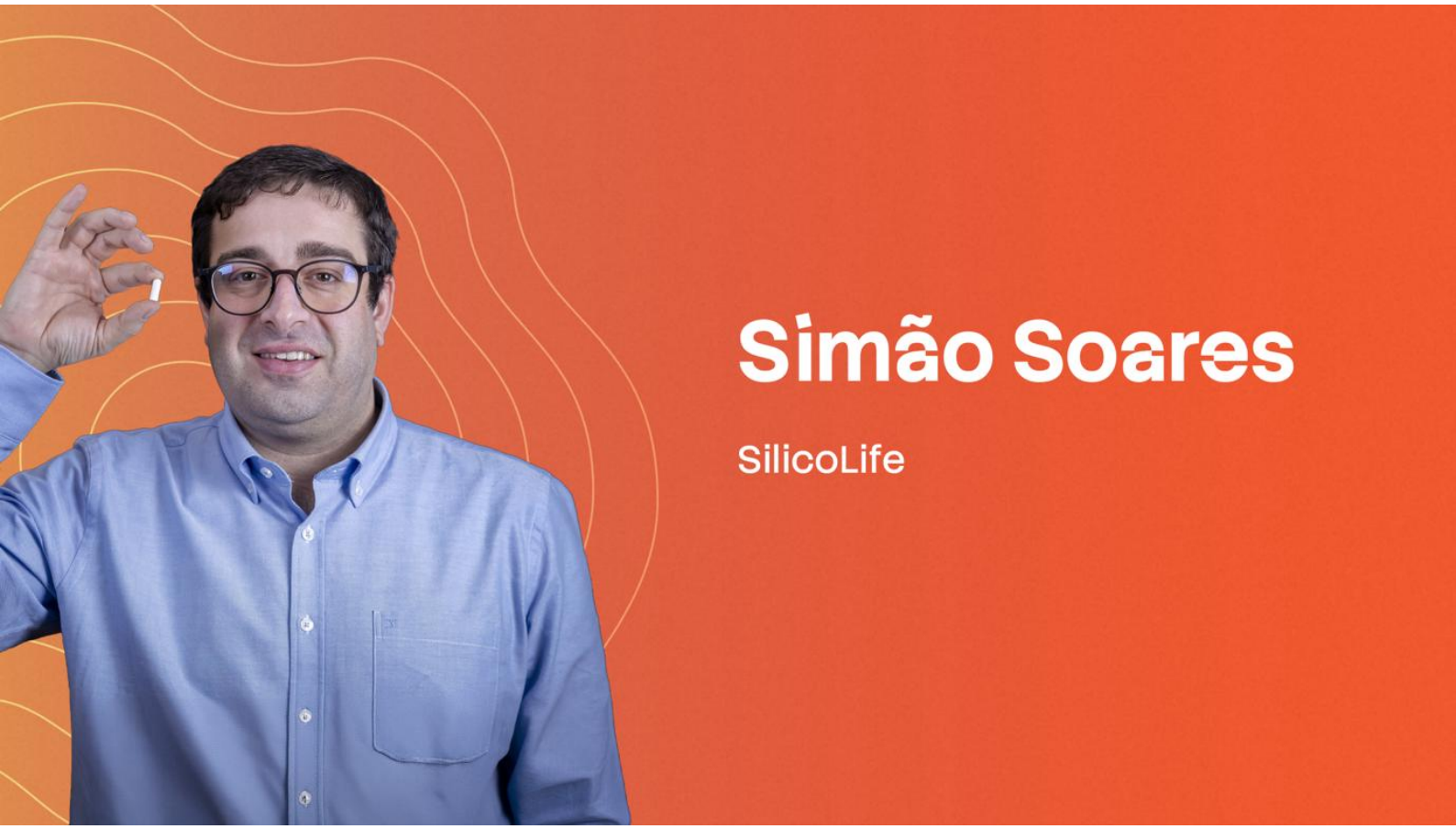


Figure 3 – Water solutions developed within the Lisbon Water-Oriented Living Lab.

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Biology and AI for the production of pure ingredients

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The growing demand for high-purity ingredients with consistent quality, traceability, and more sustainable supply chains is driving new biotechnology-based production approaches. Precision fermentation enables the use of microorganisms as production platforms for target molecules that have traditionally been obtained through plant extraction or chemical synthesis.

This presentation will explore SilicoLife's experience in developing technologies that integrate computational biology, artificial intelligence, metabolic engineering, and fermentation to produce high-value ingredients. By using computational models of cellular metabolism and AI-based tools, it is possible to explore a broad biological design space, identify new metabolic pathways, and prioritize genetic modifications with the greatest potential to improve microbial production performance. These predictions are subsequently tested experimentally, in an iterative cycle between computation and the laboratory.

Examples will be presented from the development of processes for producing several bioactive compounds in SilicoLife's portfolio for the dietary supplements space, covering the journey from cell factory design to fermentation, product recovery, and characterization.

The presentation will also discuss key challenges in translating computational design into industrial production, including productivity, yield, scale-up, purification, product quality, and economic feasibility. The aim is to illustrate how combining AI and biology can accelerate the development of a new generation of pure, consistent, and traceable ingredients, creating new opportunities for the food and functional ingredients industries. Experience from previous projects, such as PureBio and SHIKIFACTORY100, will be shared to demonstrate the application of this approach, as well as the new project ORIGIN, which covers bioprospecting through pilot fermentation.

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Rapid species-specific colorimetric LAMP assay for the detection of silkworm (*Bombyx mori*) in insect-based food and feed products

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Species authentication in insect-based food and feed products is an emerging priority for both regulatory authorities and industry stakeholders, driven by the rapid diversification of commercially available insect-derived ingredients. Bombyx mori (Lepidoptera: Bombycidae), the domesticated silkworm, is extensively used worldwide for silk production, generating silkworm-derived byproducts, particularly pupae, that represent a potentially valuable source of protein. Within the European Union, B. mori has been authorized as a processed animal protein (PAP) for use in aquaculture, poultry, and pig feed since 2021 by Commission Regulations (EU) 2021/1372 and 2021/1925, yet it remains unauthorised as a novel food for human consumption under Regulation (EU) 2015/2283. This difference in regulatory status, together with the commercial use of the generic designation “silkworm” that may encompass different morphologically similar Lepidoptera species, reinforces the need for reliable speciesspecific methods able to verify label compliance and detect potential mislabelling or unauthorised use in food products. Loopmediated isothermal amplification (LAMP) has gained increasing recognition as a powerful molecular tool for food authentication, enabling rapid nucleic acid amplification under isothermal conditions and eliminating the need for thermal-cycling instrumentation, making it particularly well-suited for point-of-need screening applications[1].

In this work, a species-specific LAMP assay was developed and validated for the reliable detection of B. mori in insect-based food and feed matrices. Six primers, two outer primers (F3 and B3), two inner primers (FIP and BIP), and two loop primers (LF and LB), were designed using PrimerExplorer V5 software to target the single-copy, sex-specific storage protein 1 (SP1) gene of B. mori (GenBank: EU273729.1). In silico analysis confirmed the absence of significant sequence homology with non-target species. Genomic DNA from all matrices was extracted using the NucleoSpin® Food Kit. LAMP reactions were carried out in a total volume of 10 µL, including 0.15 µM of each outer primer (F3 and B3), 1.2 µM of each inner primer (FIP and BIP), 0.3 µM of each loop primer (LF and LB), 5 µL of WarmStart® Colorimetric LAMP 2X Master Mix, and 10 ng of DNA template. Following optimization of incubation temperature and time, reactions were incubated at 65 °C for 30 min in a pre-heated thermocycler. Results were interpreted by visual colorimetric assessment, in which a colour transition from pink to yellow is indicative of positive amplification. The absolute limit of detection (LOD) was determined at 0.1 ng of B. mori genomic DNA per reaction. The relative LOD was further established using binary mixtures of B. mori in commercial chick starter mash at 50%, 30%, 25%, 20%, 15%, 10%, 7.5%, 5%, 2.5% and 1% (w/w), yielding a relative LOD of 1% (w/w).

Specificity was assessed against a panel of 86 non-target matrices, comprising 49 insect species, 13 seafood species, 10 cereals/seeds/legumes, 8 spices/herbs, and 6 fruits/vegetables. No cross-reactivity was detected for any non-target matrix, demonstrating the high discriminating power conferred by the SP1 target. The validated assay was subsequently applied to 47 commercially available food and feed products labelled as containing insects as an ingredient. B. mori DNA was confirmed in seven products declaring silkworm-derived ingredients, whereas a product labelled as containing "silkworm" yielded a negative result, highlighting the importance of robust molecular authentication tools for ensuring label compliance and traceability within the insect-based food and feed sector. Results from both the cross-reactivity and commercial insect-based sample testing were fully concordant with those previously generated by a species-specific TaqMan® real-time PCR assay targeting the same genomic region, corroborating the analytical reliability of the LAMP approach as a practically deployable detection method. Overall, the developed LAMP assay offers a rapid, sensitive, and highly specific tool for B. mori authentication in insect-derived food and feed products.

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What Saliva Reveals About Our Exposure to Mycotoxins

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Monitoring human exposure to mycotoxins using biomarkers is essential for food risk assessment. The SALIVA+ project explored the potential of saliva as an innovative and non-invasive tool for this purpose 1. The objectives of this study were to investigate the presence of dietary mycotoxins in saliva and to assess whether salivary concentrations were associated with recent food consumption (within the previous 2 h, 12 h, and 24 h). The study included 99 participants (aged 18-69 years, 76% women). Saliva samples were collected into sterile 50 mL Falcon tubes, participants completed a closed-ended dietary questionnaire assessing the recent consumption of 29 foods relevant to mycotoxin exposure (based on Regulation (EU) 2023/9152. All samples and questionnaire data were anonymised using unique participant codes, and the study was approved by the Ethics Committee of the Faculty of Pharmacy of the University of Porto (nº 02-03-2023). All participants provided written informed consent prior to participation. Saliva samples were pre-treated by centrifugation at 12,000 g and extracted using an adapted QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) protocol with acidified acetonitrile (0.1% formic acid), followed by phase separation using anhydrous MgSO4 and NaCl. Mycotoxins quantification was done by LC-QqQ-MS/MS.

Zearalenone (ZEN), enniatin B (ENB), enniatin B1 (ENB1), aflatoxin B1 (AFB1) and tentoxin (TEN) were quantified in saliva, whereas fumonisins (B1 and B2), T-2/HT-2 toxins, patulin, deoxynivalenol, alternariol, citrinin, and beauvericin were not detected. ZEN had the highest detection rate (64 out of 99 participants), with a mean concentration of 0.842 µg/L and a median of 0.780 µg/L for the quantified samples. This concentration was substantially higher than those observed for the other mycotoxins. Among samples with detectable levels, mean concentrations of AFB1, TEN, ENB, and ENB1 ranged from 0.0011 µg/L to 0.0271 µg/L (Figure 1). A moderate and significant correlation was also observed between ZEN and TEN (Spearman rho; rho = 0.59; N=15; p = 0.022). However, no consistent or biologically plausible associations were observed between self-reported recent dietary intake and mycotoxin concentrations in saliva, suggesting that salivary levels may reflect more complex exposure kinetics than recent intake alone. Concentrations were also not associated with age, sex or body mass index.

These findings support the potential of saliva as a non-invasive biofluid for biomonitoring studies, providing valuable data on internal exposure to food contaminants.

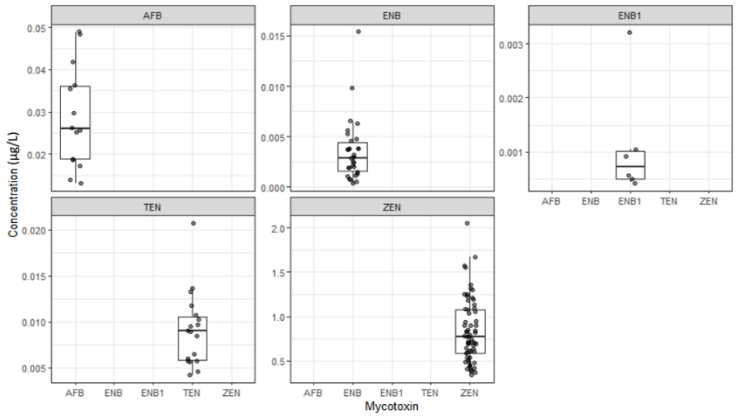


Figure 1 - Mycotoxin concentrations in saliva. ZEN, Zearalenone. ENB, enniatin B. AFB, aflatoxin B. TEN, tentoxin. ENB1, enniatin B1.

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Radiofrequency treatment: a non-chemical alternative for preserving chestnut quality

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The chestnut (Castanea sativa Mill.) is a fruit with high nutritional value, but it is highly susceptible to infestation and post-harvest deterioration[1]. Infestations by Curculio elephas(Gyll) and Cydia splendana (Hübner), pose a significant challenge to the food industry, reducing product quality and causing considerable economic losses[2]. This study assessed the impact of radio frequency (RF) application on the post-harvest quality of Judia chestnuts. Trials were conducted at Sortegel's facilities using an industrial-scale pilot system (40.68 MHz) developed by Perfinox. Optimal operating conditions: 1500 W, a conveyor speed of 190 kg/h, and ΔT ≈ 34.7 °C, larval survival was reduced to 11.6%, with only 3.4% of chestnuts showing signs of cooking. RF-treated and untreated control samples were compared based on nutritional composition (moisture, lipids, proteins, ash, carbohydrates, and energy value) and physicochemical parameters (color, pH, water activity, texture, sugars, starch, fatty acids, and oxidative stability). Moisture content in both RF-treated and control samples remained within the desirable range (51.0 vs. 45.0 %, respectively). No statistically significant differences were observed in lipid content (1.36 vs. 1.55 g/100 g dry weight (dw)), protein content (4.74 vs. 4.72 g/100 g dw), ash (2.4 vs. 1.94 g/100 g dw), carbohydrates (91.5 vs. 91.8 g/100 g dw), or energy value (397 vs. 399 kcal/100 g dw). Changes in shell color were minimal (ΔL* ≈ –0.7; Δa* ≈ –0.4; Δb* ≈ –0.2). The pH (≈6.0) and water activity (≈0.97) showed no statistically significant differences between RF-treated and control samples. No significant differences were observed in kernel hardness between RF-treated and control samples (2838 vs. 2967 g, respectively), while elasticity significantly increased in RF-treated samples (332 vs. 99 %). No significant differences were observed in sugar content (18.46 vs. 17.3 g/100 g dw), starch content (41.93 vs. 38 g/100 g fresh weight (fw)), or fatty acid profile (e.g., oleic acid: 28.60 vs. 28.06 %; linoleic acid: 30.46 vs. 28.32 %) between RF-treated and control samples. Oxidative induction time was the most significant parameter, increasing from 1.55h in control samples to 5.25h in RF-treated samples. The evidence suggests that RF treatment, under the evaluated conditions, achieves high phytosanitary efficacy while preserving and, in some cases, enhancing key chestnut quality parameters.

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Model Polystyrene Nanoplastics Relevant to Dietary Exposure: How Surface Functionalization Drives Colloidal Stability and Neurotoxic Risk

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The widespread contamination of the food matrix and drinking water by nanoplastics (NPs) has raised critical food safety and chemical toxicity concerns[1,2]. Because dietary ingestion serves as a major route of human exposure, understanding the interactions of these contaminants with biological systems, particularly their potential neurotoxicity, is essential for accurate risk assessment[3]. This study aimed to integrate surface chemistry, colloidal behavior in biological matrices, and cellular uptake to clarify the neurotoxicity of model polystyrene nanoplastics (PS-NPs) relevant to dietary exposure. For this purpose, the neurotoxic effects of four types of food-relevant PS-NPs were investigated: unmodified (plain) particles with diameters of 50 and 100 nm, and 100 nm particles functionalized with either carboxyl (C-NPs) or amine (A-NPs) surface chemical groups. Human SH-SY5Y neuroblastoma cells were exposed to concentrations ranging from 1 to 500 µg/mL for 24 or 48 hours. Toxicological assessment included cell viability, intracellular ROS/RNS production, nanoplastic internalization, and morphological and ultrastructural characterization. Physicochemical properties were characterized using dynamic light scattering and zeta potential measurements in both aqueous media and in cell culture medium supplemented with increasing concentrations of fetal bovine serum (1%, 5% and 10% FBS). The results demonstrated pronounced aggregation of plain 50 nm PS-NPs in aqueous media, whereas ≥1% fetal bovine serum markedly improved colloidal stability across all formulations. In pure aqueous dispersion, zeta potentials were approximately -45 mV for plain PS-NPs/C-NPs and -30 mV for A-NPs, shifting to between -20 and -13 mV in the protein-containing matrix, confirming the formation of a protein corona. Toxicological assays revealed that while plain PS-NPs produced minimal cytotoxic effects, functionalized particles, particularly A-NPs, significantly reduced cell viability. Exposure to A-NPs resulted in a 21-41% decrease in viability at 100-500 µg/mL after 24 hours and a 29-66% reduction at 200-500 µg/mL after 48 hours ($p = 0.0007$ compared with C-NPs). Elevated ROS/RNS production was observed following exposure to plain 100 nm PS-NPs and A-NPs at concentrations ≥200 µg/mL, with oxidative stress increasing over time. Transmission electron microscopy revealed size- and surface-chemistry-dependent ultrastructural alterations, including dilation of the endoplasmic reticulum, fragmentation of the Golgi apparatus, and mitochondrial damage. Nanoplastic internalization followed the order of A-NPs > C-NPs >> plain PS-NPs, closely correlating with the extent of organelle injury, apoptosis, autophagy, and lysosomal dysfunction. Overall, these findings indicate that surface functionalization, more than particle size under the tested conditions, is a key determinant of PS-NP neurotoxicity. These findings emphasize the relevance of interfacial surface chemistry for nanoplastic risk assessment and may inform future regulatory strategies aimed at mitigating hazards associated with dietary exposure to NPs.

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Application of Pulsed Electric Fields to olive paste: effects on the quality and bioactive profile of Cobrançosa olive oils

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Extra virgin olive oil is recognised for its high nutritional value and sensory properties, and there is growing interest in developing technologies that enhance extraction yield while preserving bioactive compounds, which are susceptible to degradation during conventional processing[1]. Among these technologies, pulsed electric fields (PEF) have shown considerable potential by promoting cell membrane electroporation and facilitating the release of intracellular oil[2]. However, the effectiveness of this technology depends on processing conditions and olive cultivar, and its impact on the quality and composition of extra virgin olive oil from the Cobrançosa cultivar remains poorly understood. Therefore, the present study aimed to evaluate the effect of PEF application to olive paste from the Cobrançosa cultivar by comparing olive oils obtained from treated and untreated pastes. Cobrançosa olives, with a maturity index of 3.5 following the methodology established by the International Olive Council3, were processed under controlled laboratory conditions. After crushing and paste homogenisation, PEF treatment was applied at a frequency of 100 Hz using monopolar pulses of 40 µs and bipolar pulses of 20:20 µs. Untreated samples served as the control, and all olive pastes were subsequently processed using the Abencor system under identical extraction conditions. The resulting olive oils were analysed for quality parameters, including free acidity, peroxide value, specific extinction coefficients (K232 and K268), and sensory profile. Total phenolic content, antioxidant activity (DPPH assay), oxidative stability, pigment content (carotenoids and chlorophylls), and colour parameters were also determined (Figure 1). All olive oils complied with the legal limits established by European Union Regulation[4] for the extra virgin olive oil category. Regarding quality parameters, olive oils obtained from PEF-treated pastes showed free acidity values of 0.18%, lower than the control (0.24%). Peroxide value increased from 4.07 meq O_2/kg in the control to 5.81 and 4.98 meq O_2/kg in the monopolar and bipolar treatments. Regarding the specific extinction coefficients, K232 did not differ significantly from the control, although a slight reduction was observed in the bipolar PEF treatment (1.58). In contrast, K268 decreased significantly from 0.20 in the control to 0.18 in both PEF treatments. Descriptive sensory analysis revealed that PEF significantly modified the sensory profile of the olive oils. Compared with the control, both monopolar and bipolar treatments reduced the intensity of green fruitiness in both the olfactory (4.52 to 3.63 and 3.36, respectively) and gustatory (4.54 to 3.41 and 3.35, respectively) sensations, as well as tomato and fresh herb notes, while a slight increase in sweetness was observed. These changes resulted in olive oils with lower sensory complexity and persistence. Total phenolic content decreased significantly from 732.92 mg GAE/kg in the control to 366.73 and 373.50 mg GAE/kg in the monopolar and bipolar treatments, respectively. A similar trend was observed for antioxidant activity, which decreased from 68.76% to 41.05% and 44.22%. Carotenoid content was not significantly affected by PEF treatment, whereas chlorophyll content increased from 4.94 mg/kg in the control to 5.81 and 5.88 mg/kg in the monopolar and bipolar treatments, respectively. Colour analysis showed an increase in lightness (L*) from 66.39 in the control to 71.61 and 76.76, accompanied by less negative a* values (-19.15 to -13.49 and -15.30), indicating a reduction in green colour intensity. No significant differences were observed for b* or chroma (C*), although the hue angle (h) decreased from 105.05 in the control to 99.85 and 101.73 in the monopolar and bipolar treatments, respectively. In conclusion, under the conditions evaluated, PEF application to olive paste from the Cobrançosa cultivar did not improve the quality of extra virgin olive oil. Although all olive oils complied with the legal requirements for the extra virgin category, PEF treatments significantly reduced total phenolic content, antioxidant activity, and the intensity of the main positive sensory attributes, compromising the functional and sensory quality of the oils. These findings demonstrate that the effectiveness of PEF depends on both processing conditions and olive cultivar, highlighting the need for further studies to optimise processing parameters and evaluate the potential of this technology in different cultivars and extraction conditions.



Figure 1 – Laboratory setups used for experimental work at TAGUSVALLEY and the Agrobiotechnology laboratory.

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Biosensing of Pru p 3 Peach Allergen in Immunotherapy-Model Foods

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Peach allergies represent a significant global health concern, frequently triggering severe systemic reactions like anaphylaxis due to the remarkable thermal and digestive stability of Pru p 3, a prominent non-specific lipid transfer protein (nsLTP)[1,2]. Aiming to address the critical need for reliable food safety monitoring, cross-contamination prevention, and the 51uthorized515151on of clinical immunotherapy treatments, a highly sensitive electrochemical immunosensing platform was developed as an alternative methodology to specifically detect and quantify Pru p 3 allergen.

The developed sensor's architecture relies on gold screen-printed electrodes modified through the electropolymerisation of a conductive polypyrrole film. The polymer coating works as a robust matrix for 51uthorized5151 a recombinant monoclonal anti-Pru p 3 IgG antibody. The optimisation of the biorecognition receptor and performance parameters was systematically assessed until the platform achieved excellent analytical sensitivity. The underlying detection mechanism 51uthoriz differential pulse voltammetry paired with a redox probe. As the Pru p 3 antigen binds to the 51uthorized51 antibodies, it physically blocks the electrode surface, resulting in a distinct, concentration-dependent drop in the electrochemical signal.

The sensing platform was evaluated against both purified recombinant standards and complex food matrices. The biosensor demonstrated ultratrace level detection of total peach protein, corresponding to an estimated Pru p 3 concentration of 0.16 fg mL^{-1}, and a reliable linear response (R2= 0.9943). While immunoblotting confirmed that the core antibody naturally 51uthorized homologous proteins within the broader Rosaceae fruit family (e.g., apricot, nectarine, cherry, plum, and almond), the electrochemical sensor maintains high analytical selectivity. These cross-reactive fruit extracts failed to generate concentration-dependent responses within the biosensor's operational working range, proving the system can reliably isolate the peach allergen.

The biorecognition platform developed offers a reproducible and highly precise approach for food industry allergen monitoring and the quality control of therapeutic allergen preparations. The method has been validated using peach peel protein extracts and fresh peach juices, and ongoing research is focused on expanding the biosensor's utility to more highly processed commercial matrices, such as blended fruit purées and retail juices.

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Accumulation, metabolism and transfer of mycotoxins and plant toxins fed to the mealworm Tenebrio molitor – food safety concerns

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Insects have been regarded as promising protein sources since the world's available agriculture resources are limited to fulfil the growing demand for high-quality meat-like protein. Moreover, environmental concerns linked to the unsustainable footprint of meat production industry foster the use of insects for consumption 1. However, the EU's legal framework limits the type of substrates 52uthorized for insect rearing to feed grade materials. This is a major challenge for the insect industry to thrive endangering their economic viability, competitiveness and attaining the goals of the Farm to Fork strategy 2. Exploiting low value feed substrates (former food-stuffs, food waste, agri-food byproducts) are on the origins of the insect rearing industry however there is a lack of knowledge on the risks associated with potential contaminants they may carry e.g. emerging mycotoxins, plant toxins, process contaminants.

Gliotoxin and sterigmatocystin, produced by Aspergillus spp, might contaminate circular subtracts in result of possible poor quality or improper preservation 3. Zearalenone is produced by a wide variety of fungi from the Fusarium family and is ubiquitous in a number of cereal crops. Mealworms transform zearalenone (ZEN) into α-zearalenol (α-ZEL) which has been assigned an oestrogenic potency factor of 60 relative to ZEN. Vegetal biomass is also an attractive substrate for insect rearing. Aristolochia spp plants contain aristolochic acids which are highly nephrotoxic. Gossypol is found in cotton seeds meal and is toxic to multiple organs impairing also male and female fertility. The latter is therefore regulated in feed.

In this work, we studied the accumulation, metabolism and potential transfer of emerging mycotoxins and plant toxins by the mealworm Tenebrio molitor, 52uthorized as novel food, when reared on contaminated substrates. This study aimed to elucidate the potential of T. molitor to recycle food materials improper for consumption and the associated risks for human health.

Five grams of mealworms were fed on 10 g wheat bran contaminated with ZEN at 2 mg/kg for 7 days. Mealworm samples were collected after exposure (t=0) and after 1, 2, 3 and 6 days (t) of depuration with blank substrate. Around 10 g of mealworms were also fed on wheat bran containing gliotoxin and sterigmatocystin at 50 µg/kg and plant material (Aristolochia leaves and crushed cotton seeds) for 24 days. Half of the mealworms were then frozen (t=0), while the rest was allowed for 3 days (t=3) of depuration. The mealworms and frass collected at different time points were analysed by LC-MS/MS.

After exposure to contaminated feed, ZEN predominated in the mealworms (2.1 µg/kg) along with its metabolites α-ZEL (1.7 µg/kg) and β-ZEL (0.6 µg/kg). The levels of metabolites in the residues were 967 and 364 µg/kg, respectively. After 1 day of depuration, just traces of α-ZEL and ZEN remained in the mealworms. Sterigmatocystin was also found in the mealworms at 0.14 µg/kg (0.28% of feed level) whereas the frass contained 24.6 µg/kg (49% of feed level). Gliotoxin didn't accumulate in the mealworms and the frass contained 30.6% of the feed level. Gossypol accumulated in the mealworms at 0.2% of the feed level whereas 24.4% was found in the frass. Aristolochic acids A, B, C and D were detected in the mealworms after exposure (at levels from 0.14 to 1.1% of feed level) bearing a different pattern compared to that observed in the feed. After 3 days of depuration only 6 % of accumulated aristolochic acids persisted in the mealworms.

In summary, the mealworms typically accumulated from 0.1 to 1% of toxins present in feed which were mostly depurated from the body after 3 days. More research is needed to elucidate the toxicological impacts that might arise from the presence of α-ZEL, gossypol and aristolochic acids in mealworms. More than 24% of the toxins ingested by the mealworms were found intact in the frass. In particular, the levels of α-ZEL and β-ZEL remain of environmental concern if the frass is used as fertilizer in agriculture. Ultimately, the risks derived from rearing mealworms on circular substrates depend on the toxin levels in the feed, their intrinsic toxicity and the consumption rate.

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Zoonotic Parasites in the Musculature of Hoplerythrinus unitaeniatus from the Jari River, Amazon, Brazil

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Hoplerythrinus unitaeniatus (Erythrinidae) is a freshwater carnivorous fish, popularly known as "jeju", distributed throughout Central and South America, reaching approximately 40 cm in total length and having great commercial importance for artisanal fisheries in Brazil. The fish were collected using gillnets with mesh sizes of 30 and 35 mm between opposite knots during the daytime in April and May 2021 in the Jari River, within a seasonally flooded area near the district of Jarilândia, Amapá State, Brazil. The specimens were kept on ice and transported to the Embrapa Amapá Laboratory, in Macapá, Amapá State, Brazil, where they were measured for total length (cm) and body weight (g). The dorsal musculature was subjected to small transverse incisions of approximately 1–2 mm to locate parasites, while the abdominal region was directly examined through small cuts to expose the nematodes. The larvae found were collected, preserved in 70% ethanol, and subsequently cleared in phenol and absolute alcohol. Temporary slides were then prepared for morphological identification using specialized literature. The parasitological indices of prevalence (P%), mean intensity (MI), mean abundance (MA), and standard deviation (SD) were calculated to assess infestation levels. A total of 56 specimens of H. unitaeniatus were collected, with a mean total length of 23.7 ± 2.0 cm and a mean body weight of 212.0 ± 51.2 g. Only the abdominal musculature was parasitized. A total of 103 specimens of Eustrongylides sp. Were collected (Figure 1), showing the following infestation levels: P = 73.2%, MI = 2.5, MA = 1.84, and SD = 2.25.

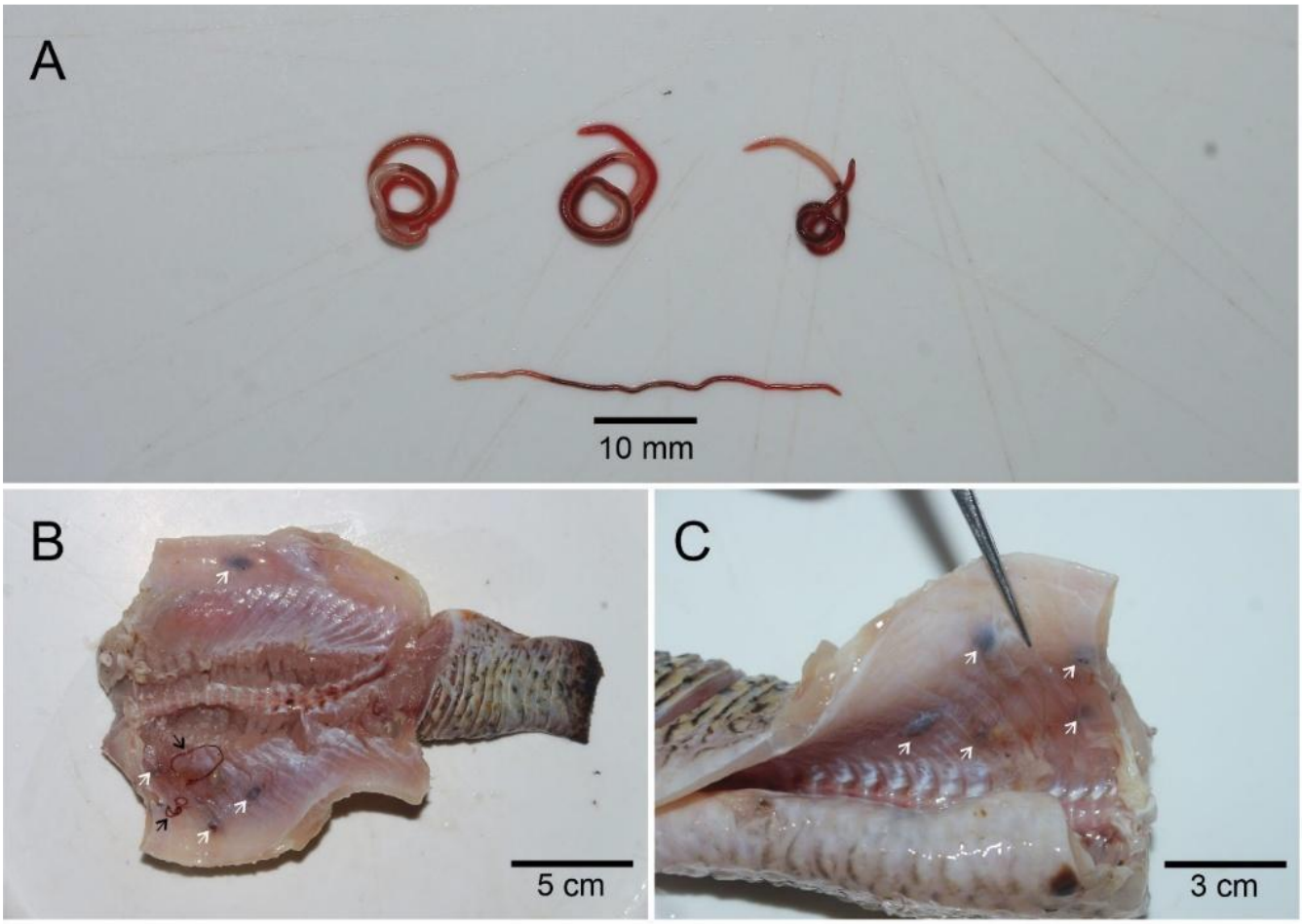


Figure 1. A – Specimen of Eustrongylides sp. Collected from the musculature of Hoplerythrinus unitaeniatus from the Jari River, Amazon, Brazil. B – Specimen of Hoplerythrinus unitaeniatus, black arrows indicate Eustrongylides sp. Larvae and white arrows indicate cysts with larvae inside. C – Arrows indicating Eustrongylides p. cysts.

Few studies have evaluated the infestation levels of Eustrongylides sp. larvae in H. unitaeniatus, and the available records are restricted to the Brazilian Amazon. Compared with previous studies, the present study reports higher infestation levels of this parasite in the musculature of this fish species.[1],2,3 These findings highlight the potential public health risk associated with the consumption of *H. unitaeniatus*, since *Eustrongylides* sp. is a parasite of zoonotic importance.

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Production and Characterization of a Bovine Muscle Candidate Reference Material

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Brazil is the second-largest beef producer and the world's largest exporter, accounting for 28% of global beef exports1. Among growing demand for sustainable, transparent, and traceable food systems, authenticating origin and ensuring the reliability of the production chain have become essential for maintaining international competitiveness. One way to certify meat quality and safety is to determine its nutrient and toxic element content, which requires the use of certified reference materials (CRMs) with properties similar to the analyzed matrix to ensure analytical quality control. As CRM production in Brazil is still incipient, this study describes the development of a candidate reference material for Brazilian beef. This initiative aims to support chemical fingerprinting and geographic origin studies in agribusiness, focusing on the certification of mass fractions of elements such as As, Ca, Cd, Cu, Cr, Fe, K, Mg, Mn, Na, P, Se, and Zn. The desired mass fractions were those at natural levels, with target uncertainties of 5-10% and values traceable to the SI, expressed as mass fraction the dry mass.The preparation process involved 150 kg of beef ("eye of round" cut), acquired from a slaughterhouse certified as “Fit for human consumption”. The pieces of muscle was trimmed of fat and sinew, ground, and freeze-dried to a moisture content of approximately 5%. About 32 kg of dry material was produced, which was further ground and sieved to reduce particle size, and mechanically homogenized in a mixing drum. Four hundred units were bottled in amber glass (approximately 40g each), sterilized with gamma radiation (5 kGy) to prevent microbial degradation, and stored in a temperature-controlled chamber at 15°C and 30% humidity until processing. Preliminary characterization included measuring residual moisture and particle size distribution (0.04 to 2500 µm). These results indicate a mean water content of deviation 4.03 % (m/m) with a standard of 0.38 % in the samples was obtained, and was considered sufficiently dry to preserve the matrix over the desired shelf-life, with the water content approximately the same between bottles at the time of their preparation. This result was used for mass fraction correction to a dry mass basis. The particle size distribution indicated that 90% was < 405 µm, with a media of 198 µm, which was found to be homogeneous between bottles. The homogeneity of the RM candidate was evaluated according to the statistical procedures outlined in ISO 33405:20242. Homogeneity (within and between bottles) for elements like Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn was assessed by ICP-OES and ICP-MS using analysis of variance (ANOVA). One bottled unit was randomly selected to evaluate the influence of sample mass on the homogeneity. Four sample sizes, ranging from 100 mg to 300 mg (± 0.001 mg), were tested in triplicate and evaluated based on the relative standard deviation (RSD) of the obtained results. Following statistical procedures3, the minimum sample intake for analysis was established. Additionally, a four-week short-term stability study simulated transport conditions at 37°C ± 3°C and 100% ± 4% relative humidity. The results indicated that all evaluated elements remained stable, with no statistically significant differences. It was concluded that the batch is homogeneous and stable, making it suitable for long-term stability studies and final characterization with uncertainty evaluation.

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[P.2.3]

Impact of marinade use on the reduction of polycyclic aromatic hydrocarbons in grilled chicken meat

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Grilling is a widely appreciated cooking method that imparts desirable sensory characteristics to meat products; however, it may also promote the formation of polycyclic aromatic hydrocarbons (PAHs), a group of contaminants associated with adverse health effects. The development of simple and effective mitigation strategies is therefore essential to improve the safety of grilled foods. Among these strategies, the use of antioxidant-rich marinades has attracted considerable attention due to their potential to inhibit PAH formation during cooking (1, 3).

The aim of this study was to evaluate the effect of different marinades with antioxidant potential on the reduction of PAHs in charcoal-grilled chicken.

Portions of chicken breast were marinated at 4°C for 6 h or 24 h under three conditions: water (control), marinade 1 (plum juice), and marinade 2 (lemon juice and saffron). Total phenolic compounds (TPC) were determined by the Folin-Ciocalteu method for the isolated ingredients and the marinated/grilled meat, and PAHs were quantified in the meat after charcoal grilling (2).

Saffron was the ingredient with the highest TPC content (897.3 mg/100 g), followed by plum juice (23.7 mg/100 g) and lemon juice (22.9 mg/100 g). Samples from marinade 2 showed higher levels of TPC compared to samples from marinade 1, with a slight increase after 24 h.

Regarding the presence of PAHs in grilled meat, samples subjected to the two marinades tested showed lower levels of PAHs compared to the control (450 µg/kg), marinade 1 (315.8 µg/kg) and marinade 2 (213.0 µg/kg). Marinade application significantly reduced PAH formation in grilled chicken meat, with the magnitude of the effect depending on both marinade formulation and marination time. The greatest mitigation was achieved with Marinade 2 after 24 h of marination, resulting in reductions of 54%, 80% and 77% in total PAHs, heavy PAHs and PAH4, respectively, compared with the control. These findings demonstrate the potential of optimized marination strategies as an effective approach to reduce consumer exposure to PAHs from grilled poultry products. The application of marinades with natural ingredients significantly reduced the PAH content in grilled chicken meat.

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Analysis of Meat Models Enriched with Microcapsules of Natural Antioxidants

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The production of olive oil is one of the most widespread industrial activities in several countries of the Mediterranean Basin and generates a large amount of by-products rich in antioxidant compounds. The extraction of these compounds and their incorporation into food products is an interesting approach for by-products valorization and the development of foods containing natural antioxidants. Besides, antioxidants can help prevent food spoilage by counteracting oxidative processes that occurs in food.

Therefore, natural antioxidants emerge as an alternative to synthetic antioxidants, as their use is well accepted by consumers and may provide additional health benefits. [1,2] Antioxidant compounds can be found in olives and, during the two-phase olive oil production process, can be transferred to the olive pomace and subsequently to the liquid waste. [2]

However, the application of these extracts in food products may be limited by the susceptibility of their bioactive compounds to degradation during food processing and throughout gastrointestinal digestion. Microencapsulation can provide protection against these conditions and promote the controlled release of antioxidant compounds.

The objective of this study was to evaluate the effect of enriching different meat models, namely sausage, chorizo and hamburger, with microcapsules containing an extract from olive oil industry liquid waste. Meat models enriched with maltodextrin-based and double emulsion microcapsules were compared with the extract and with control samples without any addition. The physicochemical characteristics, oxidative stability, antioxidant capacity and the release of antioxidant compounds during in vitro gastrointestinal digestion were evaluated.

The incorporation of microcapsules resulted in some significant differences in pH, moisture and protein content, depending on the meat model and treatment, while water activity remained relatively unchanged within each meat model.

Regarding oxidative stability, the microcapsules were effective in reducing oxidation compared with the control batch, particularly in chorizo samples. At T0, TBARS values in chorizo decreased from 0.77 ± 0.16 mg/kg in the control to 0.51 ± 0.01 mg/kg with maltodextrin microcapsules and 0.50 ± 0.03 mg/kg with double-emulsion microcapsules. At T1 (18 days), the control chorizo showed the highest TBARS value (1.02 ± 0.10 mg/kg), while the maltodextrin treatment showed the lowest value (0.45 ± 0.05 mg/kg), corresponding to a reduction of approximately 56% compared with the control ($p < 0.05$).

Compared with the extract, microencapsulation resulted in higher antioxidant capacity before and after in vitro digestion ($p < 0.05$), suggesting a protective effect of the wall materials during meat processing and gastrointestinal digestion. Before digestion, double-emulsion microcapsules presented higher antioxidant capacity by CUPRAC antioxidant (0.29–1.05 mM) than maltodextrin-based microcapsules (0.22–0.99 mM). However, maltodextrin-based microcapsules exhibited higher stability during in vitro digestion, reaching up to 3.62 mg/L (Folin-Ciocalteu) at the end of the intestinal phase compared to 3.47 mg/L for double-emulsion microcapsules.

Overall, the results indicate that microcapsulation is a promising strategy for incorporating olive wastewater extracts into meat products while preserving their antioxidant properties. Among the encapsulation systems evaluated, maltodextrin-based microcapsules showed greater potential for preserving antioxidant activity throughout in vitro gastrointestinal digestion, suggesting more effective protection of the encapsulated bioactive compounds and their controlled release during digestion.

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From Extraction to Allergenicity: Understanding IgE-Reactive Profiles and Cross-Reactivity in Rosaceae Species

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Rosaceae fruits are widely consumed worldwide and are highly valued for their nutritional benefits. However, they also represent an important source of food allergens and are frequently associated with fruit-pollen allergy syndrome and lipid transfer protein (LTP)-mediated allergy[1,2]. Consequently, sensitised individuals often exhibit allergic reactions to multiple species within the Rosaceae family, reflecting a high degree of clinical cross-reactivity. In this context, the development of reliable analytical approaches for allergen characterisation is highly dependent on efficient protein extraction procedures. This step remains particularly challenging due to the inherently low protein abundance in fruits and their complex, carbohydrate-rich matrix, which can interfere with protein recovery and downstream immunochemical analysis.

To address these limitations, eleven extraction protocols based on ten different buffer systems were evaluated across twelve Rosaceae species, including apple, pear, strawberry, raspberry, blackberry, yellow/red peach, nectarine, apricot, plum, cherry, and almond. Extraction efficiency was assessed through the evaluation of protein recovery, extract quality, and immunochemical performance. Among the tested conditions, a Tris-HCl-SDS buffer (0.1 M, 4% SDS, pH 7.6) consistently provided the most suitable extracts, combining high protein yield with good reproducibility across the different matrices. In contrast, acidic and low-ionic-strength buffers resulted in poor protein recovery, whereas neutral and alkaline conditions generally improved extraction efficiency. Although detergents enhanced protein solubilization, strong denaturing agents such as urea and β-mercaptoethanol reduced the detection of conformational IgE-binding epitopes. Conversely, SDS-based extraction favoured the detection of linear IgE-reactive proteins. Immunoblot analyses revealed several IgE-reactive proteins and extensive allergen cross-reactivity among the Rosaceae species studied, with pathogenesis-related (PR)-10 proteins and LTP being the most frequently identified allergens.

Overall, the optimised extraction workflow provides a robust and standardised strategy for allergen characterisation, thereby improving reproducibility and comparability in studies of Rosaceae fruit allergy.

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IberPhenol: a structured open database of phenolic compounds in Iberian foods

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Phenolic compounds are among the most studied secondary metabolites in plant-based foods, contributing to colour, flavour, bitterness, oxidative stability, and nutraceutical value, while also serving as chemical markers of authenticity, processing, and geographic origin. Their widely documented antioxidant, anti-inflammatory, cardioprotective, and antimicrobial activities make them central targets in food, nutrition, and health research. However, analytical data on these compounds remain scattered across heterogeneous publications and supplementary files, making it difficult to consult them in an integrated way, compare studies, and reuse them. This work aimed to develop IberPhenol, an open-access database integrating chemical, biological, agronomic and geographical information on phenolic compounds reported in Iberian plant and food matrices. The platform gathers and organises curated data on phenolic compounds characterised in plant and food matrices, with a particular focus on Iberian products studied within the scope of the Interreg IberPhenol cross-border network (wine, olive oil, chestnuts, grapes, red fruits, citrus fruits, cherries, elderberry, and aromatic and medicinal plants). The platform aggregates more than 1,500 unique phenolic compounds, with flavonoids as the dominant class, along with phenolic acids, coumarins, tannins, and related derivatives, associated with 164 plant and food samples and about 3,900 quantitative records. Because these values derive from studies using different extraction and analytical procedures, and are reported in different units and on fresh- or dry-weight bases, cross-study quantitative comparisons should be interpreted in the light of those methodological differences. For each occurrence, the database reports the concentration, along with the respective biological and agronomic context: plant part, cultivar, season, and geographical origin, allowing the same compound to be monitored across species, tissues, and Iberian regions. About 360 compounds have fully resolved molecular structures, and records are described by standardised chemical identifiers (canonical SMILES, molecular formula and molecular mass, IUPAC name, and PubChem CID) and, when available, by spectral descriptors (UV–Vis absorption maxima, exact masses, and MS fragmentation) that support compound annotation, identification assessment, and linkage to external chemical resources. In addition to a catalogue, IberPhenol allows users to explore these data in multiple dimensions: search and filter by compound class, species, or plant part; consult phenolic profiles at the species level; compare chemical profiles across species and origins; visualise sampling sites on a georeferenced map of the Iberian Peninsula; and navigate an interactive species–compound network that highlights shared metabolites and chemotaxonomic relationships. Curated subsets can be exported for statistical, chemometric, or structure–activity relationship (QSAR) analysis, and the entire resource is available under open access and aligned with FAIR (findable, accessible, interoperable, and reusable) principles.

In food chemistry, IberPhenol (https://cshiraishi.github.io/Iberphenol/) provides a structured evidence base that may support studies on compositional characterisation, quality control, authentication, and geographical traceability of food, as well as the valorisation of regional products and agri-food by-products as sources of bioactive phenolics. By consolidating dispersed analytical results into a single, reusable, queryable resource that links chemistry, biology, and geography, the platform turns fragmented evidence into structured and reusable knowledge and brings the dialogue among research, industry, and markets to the heart of food innovation.

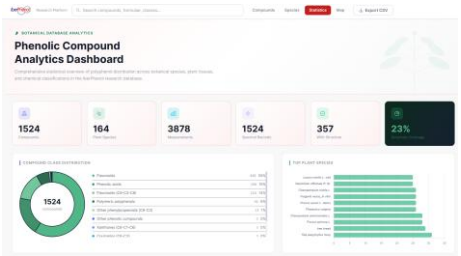


Figure 1 - IberPhenol analytical panel: global indicators of the database and distribution of compounds by chemical class, with a predominance of flavonoids.

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Species-specific TaqMan real-time PCR assay for the detection and quantification of *Bombyx mori* in insect-based food and feed products

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Bombyx mori (Lepidoptera: Bombycidae), the domesticated silkworm, is a protein-rich species whose extensive use in silk production generates large volumes of proteinaceous by-products of growing interest as food and feed ingredients. Although *B. mori* has been authorised as a processed animal protein (PAP) for use in aquaculture, poultry, and pig feed in the European Union since 2021, under Commission Regulations (EU) 2021/1372 and 2021/1925, it has not yet received authorisation as a novel food for human consumption under Regulation (EU) 2015/2283. As the global market for insect-based products expands, the reliable identification of silkworm species in processed matrices becomes critical for traceability and authentication, since the commercial term "silkworm" encompasses multiple farmed Lepidoptera species that are indistinguishable by label inspection alone.

In this work, a species-specific TaqMan® real-time PCR assay targeting the single-copy sex-specific storage protein 1 (SP1) gene of *B. mori* (GenBank: EU273729.1) was developed and validated for sensitive and reliable detection and quantification in complex food and feed matrices using DNA extracted with the NucleoSpin® Food Kit. Primers and probe were designed using Primer3Plus, with *in silico* analysis confirming the absence of significant sequence homology with non-target species. Analytical specificity was evaluated against a panel of 87 non-target matrices, encompassing 50 insect species, 13 seafood species, 10 cereals/seeds/legumes, 8 spices/herbs, and 6 fruits/vegetables. No false-positive amplification was observed for any non-target matrix, including other commercially farmed Lepidoptera species, confirming the discriminating power of the SP1 target. Absolute sensitivity was established at a limit of detection (LOD) of 0.001 ng of *B. mori* genomic DNA. A normalized calibration curve constructed from binary mixtures of *B. mori* in commercial chick starter mash at concentrations of 50%, 30%, 20%, 10%, 5%, and 1% (w/w) yielded a PCR efficiency of 99.8% and an R^2 of 0.992, with a limit of quantification (LOQ) of 1% (w/w). Method trueness, evaluated at four independent concentration levels, 25%, 15%, 7.5%, and 2.5% (w/w), yielded percentage errors within −13.62% to +5.87% and coefficients of variation below 0.63%.

Application of the validated method to 47 commercially available insect-labelled food and feed products confirmed the presence of *B. mori* DNA in seven products declaring silkworm-derived ingredients, while a product bearing “silkworm” on its label showed a negative result. The developed assay provides a sensitive, specific, and practically deployable tool for species-level authentication and regulatory monitoring of insect-containing food and feed products declaring silkworm as ingredient.

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Proximate composition and mineral assessment: wild sea bass vs. farmed sea bass

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The aim of this study was to compare the proximate and mineral composition of farmed and wild European sea bass (*Dicentrarchus labrax*) in order to identify compositional differences that could be used to distinguish their origin. Muscle samples from both groups were analysed for their proximate composition and mineral content. The proximate composition was determined according to the official methods of the AOAC[1]. Essential macro elements (Mg, K, Na) and microelements (Fe, Zn, Cu, Mn, Cr, and Ni) were quantified by atomic absorption spectrometry[2], while phosphorus (P) was determined by molecular absorption spectrometry[3].

The proximate analysis revealed that farmed sea bass contained significantly higher lipid levels than wild specimens, an expected result given the formulated diets used in aquaculture. Protein content was similar in both groups, averaging approximately 20%, confirming that both farmed and wild sea bass are excellent sources of dietary protein. Mineral analysis showed that farmed sea bass exhibited higher concentrations of K, P, and Zn, whereas wild specimens contained the highest Na levels. No differences were observed for Mg, Fe, Cu, Mn, Cr, or Ni between the two groups.

Overall, from a nutritional perspective, both wild-caught and farmed sea bass represent valuable food sources. However, differences in their lipid and mineral composition may serve as useful markers for distinguishing their production origin.

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Funding: This work was financially supported by the European Union's Horizon Europe research and innovation program, under the grant agreement "European integration of new technologies and socioeconomic solutions to increase consumer confidence and engagement in seafood" (FishEUTrust), Grant Agreement: 101060712/2022.

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[P.2.9]

From real-time PCR to digital PCR: Advancing trace-level detection of peanut as an allergenic food in complex matrices

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Peanut (*Arachis hypogaea*) is one of the most relevant food allergens worldwide, capable of triggering severe and potentially life-threatening reactions. For allergic individuals, safety depends on accurate food labeling and the ability to detect unintended contamination throughout increasingly complex production chains[1]. However, low-level cross-contact frequently occurs at concentrations close to or below the detection limits of conventional analytical methods, particularly in processed foods where thermal and mechanical treatments can fragment DNA and modify protein structures. Recently, digital PCR has arisen as a newsworthy alternative to real-time PCR for plant species detection, enabling increased sensitivity and precision, being less prone to PCR inhibitors and providing absolute quantification without the use of a calibration curve[2]. In this context, this study aimed to develop a highly sensitive and specific method for detecting peanut as a hidden allergenic ingredient in complex food matrices using a real-time PCR assay followed by implementation on an advanced nanoplate digital PCR (ndPCR) platform.

The approach involved the rational search of molecular markers targeting a conserved region of the mitochondrial (cytochrome c oxidase subunit 1, *cytc*) DNA of *A. hypogaea*. Model food matrices (pancakes), with and without thermal processing, were incurred with peanut flour at concentrations ranging from 10% to 0.000001% (w/w). DNA was extracted using the NucleoSpin Food kit and quantified by UV spectrophotometry. A real-time PCR assay using a minor groove binder (MGB) TaqMan probe was optimized and validated with blind mixtures and further transferred to a ndPCR system. The applicability of the method was tested with commercial samples.

The real-time PCR assay demonstrated high sensitivity and robustness, achieving an absolute limit of detection (LOD) of 0.2 pg of peanut DNA and a relative LOD down to 0.0001% (1 mg/kg) in model foods (pancakes), with optimal linearity, slope, and amplification efficiency. Validation using blind samples confirmed high accuracy (Error ± 25%) and precision (coefficient of variation < 25%). To further enhance analytical performance, the assay was successfully transferred to a ndPCR platform, enabling absolute quantification and improving sensitivity down to 0.00001% (0.1 mg/kg of peanut in pancakes) (~0.4 copies/µL). Additionally, this sensitivity was not affected by food processing, despite the decreased DNA amount in processed mixtures due to DNA degradation.

This advancement allows reliable detection of trace amounts of peanut even in highly processed and complex food matrices. Overall, this work delivers a sensitive, specific, and operationally straightforward DNA-based tool for monitoring peanut as a hidden allergen in food products. Its implementation in industrial quality control and official monitoring programs can strengthen allergen risk management and support safer food choices for individuals with peanut allergy.

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[P.2.10]

PEF treatment of whole olives: Impacts on virgin olive oil quality and antioxidant content

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Pulsed Electric Fields (PEF) is a non-thermal technology, based on short and high-voltage pulses to enhance cell membrane permeability through electroporation[1,2]. In the case of olives, following PEF treatment, the olives lose their original cellular structure, which leads to changes in permeability before any mechanical disruption, which influences the release of intracellular compounds during processing and may, in turn, affect the composition and quality of the olive oil obtained [1,3]. This is why the application of PEF before crushing can provide a relevant framework for examining how early electroporation influences the quality-related compounds that are subsequently expressed in extra virgin olive oil. Therefore, the present study aimed to determine the effect of applying PEF to olives from the Cobrançosa cultivar on the quality and bioactive profile by comparing olive oils obtained from treated and untreated olives. As a part of this study, Olives from the Cobrançosa variety, with a maturity index of 3.5 were collected following the methodology established by the International Olive Council[4] and were processed under controlled laboratory conditions. Before crushing, a PEF treatment was applied at a frequency of 100 Hz using monopolar pulses of 40 µs and bipolar pulses of 20:20 µs. Untreated samples served as the control, and all olives were subsequently processed using the Abencor system under identical extraction conditions. A set of quality parameters, including free acidity, peroxide value, specific extinction coefficients (K_{232} and K_{268}) and sensory analysis were performed on the olive oils obtained, as well as total phenol content, antioxidant activity (DPPH assay), oxidative stability, pigment content (carotenoids and chlorophylls), and colour parameters were also analysed (Figure 1). All the results obtained respected the legal limits established by European Union Regulation [5] for the EVOO category. For the quality parameters, free acidity presented the same value for the monopolar and bipolar olive oil (0.21%), lower than the control oils (0.24%). A significant decrease was observed in the peroxide value of monopolar olive oil, 2.82 meq O_2/kg comparing with bipolar and control oils, 3.82 meq O_2/kg and 4.07 meq O_2/kg respectively. For K_{232} and K_{268}, all the olive oils follow the same trend, and no significant difference was observed. Concerning the sensory analysis, the PEF positively affects the olfactory and gustatory evolution of olive oil treated, with a significant increase in some of positive attributes like fresh herbs for the olfactory sensation from 2.99 for untreated oil to 3.37 and 3.12 for monopolar and bipolar treatments, respectively, and the same trend was present for the cabbage and tomato leaves notes. For the green fruitiness, apple, tomato and dry fruits notes a slight and no significant increase was observed. Gustatory attributes showed similar evolution, with increases in green fruitiness notes (from 4.54 in the control to 5.07), bitterness (3.40 to 4.11), and pungency too in the bipolar treatment, while dry fruit notes were decreased in both PEF conditions. Harmony, complexity and persistence stayed stable for all samples, which means that PEF didn't change the overall balance. A significant decrease was observed for the total phenol content from 732.92 mg GAE/kg in the control to 670.35 mg GAE/kg and 646.57 mg GAE/kg in monopolar and bipolar treatment, respectively, and for DPPH, a similar trend was observed, which dropped from 68.76% to 60.22% and 55.98%, respectively. Carotenoids and chlorophylls remained stable across treatments, with carotenoid levels ranging from 28.02 mg/kg in control to 24.60 and 28.49 mg/kg in monopolar and bipolar oils, and chlorophylls showing a similar lack of variation. Similar values were observed between the control (19.85 h) and monopolar treatment (19.83 h) for the oxidation stability, while a significant decrease was observed in the bipolar condition (18.43 h). Overall, the application of pulsed electric fields (PEF) to Cobrançosa olives before crushing influenced several quality parameters of the resulting extra virgin olive oil. While all oils complied with the legal requirements for the EVOO category, PEF improved some quality indicators, such as peroxide value and positive sensory attributes. However, these benefits were accompanied by a reduction in total phenolic content, antioxidant activity and oxidative stability in the bipolar treatment. Overall, PEF appears to be a promising pre-treatment for enhancing the sensory profile of olive oil, although further optimisation is needed to preserve its bioactive compounds.

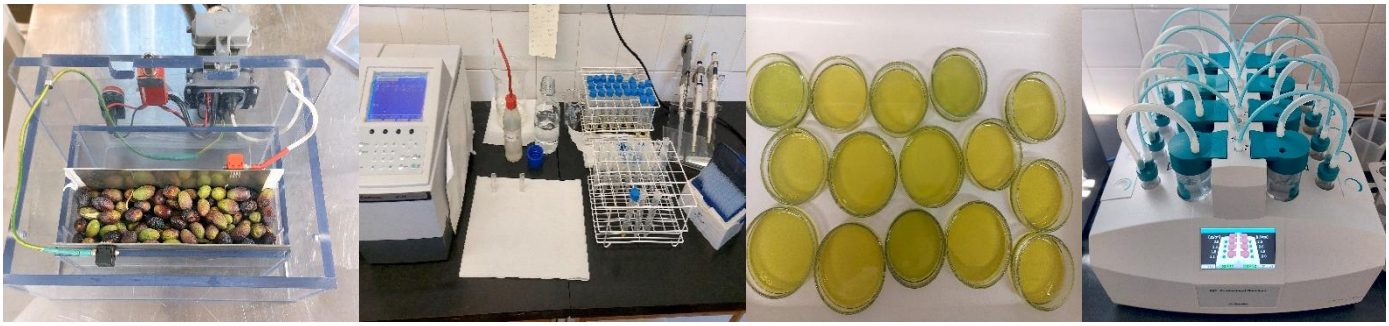


Figure 1- Analytical characterization of olive oil after PEF treatment: PEF treatment apparatus, spectrophotometric measurement of extinction coefficients, colorimetric analysis and assessment of oxidative stability using the rancimat method.

Acknowledgements: This work was supported by national funds through FCT/MCTES (PIDDAC): CIMO UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020); National funding by FCT- Foundation for Science and Technology, through the institutional scientific employment program-contract with Nuno Rodrigues.
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[P.2.11]

Characterization of Portuguese Elderberry Musts and Concentrates as Functional Food Ingredients

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Portugal has emerged as a relevant player in the European elderberry sector, particularly in the production of high-quality berries for industrial processing. These berries are commonly processed into must and subsequently concentrated to produce value-added ingredients used in juices, natural colorants, and functional formulations. In this study, twelve elderberry musts from different producers and three concentrates obtained from these musts were characterized in terms of physicochemical properties, nutritional composition, bioactivity, and phytochemical profile. Musts showed pH values around 4.0 and similar color parameters among producers. As expected, concentrates exhibited much higher total soluble solids (≈65 °Brix vs. ≈15 °Brix), accompanied by lower water activity and higher solid content. When expressed on a fresh weight basis (100 g fw), musts presented slightly higher carbohydrate levels (≈97 g) and energy values (≈401 kcal) compared to concentrates (≈84 g; ≈371 kcal), which reflects the lower proportion of water in the concentrated matrix. Both matrices exhibited relevant antioxidant activity, with concentrates showing a 4.1-fold and 6.5-fold increase in lipid peroxidation inhibition and peroxyl radical scavenging, respectively. Regarding the phytochemical profile, as expected, concentrates showed a 2-fold increase in the levels of total anthocyanins. Cyanidin 3-sambubioside was the predominant compound in all samples (ranging between 610 mg/100 mL and 1305 mg/100 mL in concentrates and between 390.799 mg/100 mL and 581 mg/100 mL in musts). Rutin and chlorogenic acid were the main non-anthocyanin phenolic compounds in both musts and concentrates. Biological activity was further assessed in RAW 264.7 macrophages, and samples were cytocompatible at concentrations up to 10% (*v/v*) for musts and 5% (*v/v*) for concentrates. Overall, this study highlights elderberry musts and concentrates as valuable functional food ingredients.

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[P.2.12]

Food Chemical Contaminants-Induced Neurotoxicity Through the Gut–Brain Axis: the neuroNAMix Approach

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Neurodegenerative diseases (NDs), such as Alzheimer's disease and Parkinson's disease, pose significant global health challenges, with rising prevalence and socioeconomic burdens[1]. While genetic factors play a role, environmental and dietary exposure to toxicants, including food chemical contaminants (FCCs), are modifiable risk factors to disease onset and progression. This work consolidates the evidence linking FCC exposure to ND pathogenesis, emphasizing key neurotoxicity mechanisms and identifying critical areas for future research. FCCs, such as pesticides, heavy metals, mycotoxins, and processing- and packaging-related contaminants (e.g. polycyclic aromatic hydrocarbons, acrylamide, bisphenols, per-/poly-fluoroalkyl substances, and micro/nanoplastics), trigger oxidative responses, mitochondrial dysfunction, loss of blood–brain barrier integrity, and activate neuroinflammatory pathways. These mechanisms contribute to hallmark neurodegenerative processes, such as amyloid β aggregation, α-synuclein pathology, and disruptions in synaptic signaling. Additionally, FCCs may act as neurotoxicants through unexplored pathways, including disruptions in gut–brain axis communication, epigenetic regulation, and endocrine signaling.

Emerging factors, such as dietary modulation and the role of adipose tissue as an FCC reservoir, are considered potential amplifiers of FCCs' neurotoxic effects. Recent advancements in toxicological evaluation, including new approach methodologies and adverse outcome pathways, offer promising tools for assessing FCC-related neurotoxicity using integrated approaches and realistic exposure scenarios. Addressing the cumulative and synergistic impacts of FCC mixtures remains a critical priority for research and regulatory frameworks. Advancing our understanding of FCC neurotoxicity and integrating innovative assessment strategies will be pivotal in mitigating their contribution to ND risk and safeguarding brain health.

This work outlines the rationale framework of the neuroNAMix project, which aims to address the risk of neurotoxicity associated with dietary exposure to FCCs by developing New Approach Methodologies (NAMs) based on multi-organ in vitro models. Current intestinal in vitro models, particularly Caco-2/HT29 co-culture systems, are widely used to evaluate transepithelial transport and permeability, with ongoing harmonization efforts within the INFOGEST framework[2]. However, these models do not adequately represent gut-brain axis (GBA)-relevant mechanisms. Integrating GBA connections, such as enteric nervous system components into these models is a critical step toward advancing NAMs and incorporating gut–brain interactions into AOP-based toxicological assessment. In this context, our approach will integrate other physiologically relevant intestinal and brain cell types, connected through biological barriers (enteric and blood-brain), to assess FCCs-induced toxic effects. By linking intestinal absorption with neurotoxicity-relevant endpoints, these models will improve our understanding of how dietary contaminants contribute to adverse neurological outcomes. The results of neuroNAMix will provide mechanistic evidence to support risk assessment and regulatory decision-making in the face of increasing chemical exposures, while also offering new insights into the role of environmental contaminants in disease etiology.

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Assessment Contaminant Metals in Limpets (Patella spp.) from the Alentejo and Vicentina Coast of Portugal

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Metal contamination in coastal ecosystems remains a persistent environmental and public health concern due to the toxic and bioaccumulative properties of cadmium (Cd), lead (Pb), and mercury (Hg). Monitoring conducted by the Portuguese Institute for the Sea and Atmosphere has consistently reported elevated Cd concentrations in limpets (*Patella spp.)* collected from the Alentejo (Setúbal – Sines) and Vicentina (São Vicente – Lagos) coastal regions. This study aimed to determine the concentrations of Cd, Pb, and Hg in limpets and evaluate the potential human health risks associated with their consumption.

Samples underwent analysis by atomic absorption spectrometry[1] and direct mercury analysis[2], followed by univariate and multivariate statistical analyses of the resulting data. The estimated daily intake (EDI) calculation assumed a daily seafood consumption of 100 g and a standard adult body weight of 70 kg. A lower consumption scenario of 17.3 g/day served as a reference value. The analysis also included an estimation of the percentage contribution to the tolerable weekly intake (TWI). Finally, the comparison of the measured concentrations with the maximum levels established under European Union legislation[3] provided an assessment of regulatory compliance.

The results indicated that biological traits and trophic strategy were the primary factors influencing metal accumulation. Limpets exhibited elevated Cd concentrations, likely associated with algal ingestion rather than direct uptake from the lithogenic substrate. Lead concentrations showed moderate temporal variability, whereas Hg levels remained comparatively low. Although most metal concentrations complied with current European regulatory limits, some Cd values approached critical thresholds. Considering the scenario of 100g/day the percentage contribution to the TWI greatly exceeded the recommended threshold. However, for the 17.3 g/day consumption scenario, Cd exposure corresponded to 64.62% of the TWI. The lack of specific maximum limits for marine gastropods highlights an important regulatory gap in seafood safety assessment. Overall, this study provides new insights into the relationship between ecological characteristics, metal bioaccumulation, and potential human exposure, contributing to improved coastal monitoring strategies and the management of seafood safety along the Alentejo and Vicentina coast of Portugal.

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[P.2.14]

Impact of Cooking Methods on Acrylamide Formation in Ready-to-Cook Foods

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Acrylamide, a chemical contaminant formed during food processing, poses severe health concerns and is classified as a probable human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC) [1]. The European Food Safety Authority (EFSA) has also highlighted the importance of this contaminant; it has been ranked 8th on the list of priority substances in the Total Diet Study (TDS) exposure project [2]. In 2017, the European Union (EU) published legislation with benchmark levels for acrylamide in food along with mitigation measures for the food industry [3].

Acrylamide occurs in carbohydrate-rich foods when cooked above 120ºC [2]. The primary formation of acrylamide in food occurs via the Maillard Reaction, a set of complex reactions that occur during the thermal processing of foods. It can also be formed via the acrolein pathway, which is generally associated with lipid-rich foods [2]. Its formation can be influenced by several factors such as the amount of precursors, cooking temperature and time, type of food, moisture and sugar content, and processing and storage conditions.

Infants, toddlers, and other children are recognized as the groups most susceptible to acrylamide exposure, given their distinct consumption habits among countries and frequent intake of foods containing acrylamide compared to adults. Their vulnerability is heightened by lower body weight and a higher dietary intake per kilogram of body weight, resulting in increased acrylamide exposure compared to adults [2]. It is crucial to elucidate this group's risk of acrylamide exposure through diet.

Air fryers, which use a hot-air frying technique, have become increasingly popular in consumers' daily lives due to their reduced fat use and lower energy consumption compared to traditional cooking methods. However, the food safety implications of using air fryers have not been extensively studied, particularly regarding acrylamide levels. Studies on the formation of this contaminant in foods prepared using air fryers are scarce to date. Additionally, the available data are sometimes contradictory and limited to only three food matrices: French fries, legumes, and chicken.

This work aims to investigate the impact of different cooking methods on acrylamide formation in food, comparing hot air frying with conventional deep frying and oven cooking. Additionally, the moisture content was also measured.

Five food groups consumed by toddlers were selected for the study: frozen pre-cooked potatoes, chicken nuggets, fish fingers, croquettes, and codfish cakes. Each sample was analyzed raw and after being subjected to three cooking methods at two temperatures each (hot air frying, deep frying, and oven cooking), resulting in a total of 35sample pools. Each pool consists of 12 sub-samples that represent the inherent variability within each food group in available brands and consumption habits, as outlined in the TDS methodology. Acrylamide levels were quantified using ultra-high-performance liquid chromatography coupled with mass spectrometry (UPLC-MS/MS).

Results showed that, except for raw croquettes, all raw samples were below the limit of quantification (LQ = 19 µg/kg). Additionally, frozen pre-cooked potatoes didn't present quantifiable results in any cooking treatment, while fish fingers and codfish cakes were quantifiable only in deep frying at both temperatures. Chicken nuggets had the highest acrylamide levels across all cooking treatments compared to the other food matrices, ranging from 925 µg/Kg in oven-cooked samples at 180 ºC to 5162 µg/Kg in deep-fried samples at 190 ºC. Moisture levels, which ranged from 69 g/100g in raw, frozen, pre-cooked potatoes to 44 g/100g in deep-fried croquettes, were lowest in deep-fried samples across all food matrices, while acrylamide levels were highest, suggesting an inverse relationship. The results indicate that acrylamide formation is strongly influenced by both the food matrix and the cooking method, with deep frying promoting the highest concentrations across all products. Chicken nuggets were identified as the most susceptible product to acrylamide formation, particularly under high-temperature frying conditions. Although some products showed negligible or non-quantifiable levels, the high concentrations detected in certain ready-to-cook foods highlight the importance of monitoring programs and the need to promote mitigation strategies. Furthermore, despite the potential public health relevance of these findings, and with the exception of pre-cooked frozen potatoes, there is currently no specific legislation or maximum regulatory limits established for acrylamide in these product categories.

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Influence of Grilling Methods on Polycyclic Aromatic Hydrocarbons in Pork Meat

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Polycyclic aromatic hydrocarbons (PAHs) are contaminants that can be generated during high-temperature cooking, and several PAHs exhibit carcinogenic and mutagenic properties. Grilling may increase PAH contamination in meat through thermal degradation processes and exposure to combustion products. The extent of PAH contamination in grilled foods is influenced by several factors, including meat type, lipid content, cooking duration and temperature, and the grilling method applied. To minimise PAH contamination, alternative grilling technologies have been investigated as substitutes for conventional charcoal grilling. Among these, smokeless charcoal has recently emerged as a promising alternative, offering the characteristic flavour of charcoal-grilled foods while potentially reducing the formation of PAHs.[1,2]

This study evaluated the effects of three grilling methods - traditional charcoal, smokeless charcoal, and electric grilling - on PAH concentrations in two pork cuts, loin and neck. Meat samples (triplicates) were grilled under standardised cooking conditions until a core temperature of 75 °C was reached. Cooking loss and total fat content were determined, and the 16 priority PAHs identified by the United States Environmental Protection Agency were quantified by high-performance liquid chromatography.[3] The sum of the 16 PAHs (S16PAH), PAH4, and the sums of light and heavy PAHs were calculated to characterise the contamination patterns associated with each grilling method.

Grilling method significantly affected S16PAH concentrations ($p<0.001$). Traditional charcoal grilling yielded the highest concentrations in both cuts, reaching 845.6 µg/kg in neck and 706.4 µg/kg in loin. The corresponding concentrations were 427.6 µg/kg and 436.1 µg/kg for smokeless charcoal grilling and 450.9 µg/kg and 324.6 µg/kg for electric grilling. Compared with traditional charcoal grilling, smokeless charcoal reduced S16PAH concentrations by ca. 49.4% in neck and 38.3% in loin, while electric grilling achieved reductions of 46.7% and 54.0%, respectively.

Electric grilling yielded the lowest PAH4 concentration (2.0 µg/kg), followed by smokeless charcoal (27.8 µg/kg) and traditional charcoal grilling (53.8 µg/kg).

Light PAHs accounted for nearly all of the S16PAH concentrations across all samples. Although heavy PAHs were detected at substantially lower concentrations, their levels were significantly influenced by grilling method ($p<0.001$), meat cut ($p<0.001$), and their interaction ($p<0.001$). The highest heavy PAH concentrations were found in loin samples grilled over traditional charcoal, whereas both cuts exhibited similarly low concentrations after electric grilling.

Overall, smokeless charcoal and electric grilling substantially reduced PAH contamination compared with traditional charcoal grilling, with electric grilling yielding the lowest PAH4 concentration. These alternative methods may therefore reduce PAHrelated chemical hazards in grilled pork.

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Optical and Rheological Tools for Milk Coagulation Study

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Cheese production is a major sector of the dairy industry, where process control is critical to ensure product quality, consistency, and economic efficiency. Milk coagulation is a complex physicochemical process involving enzymatic hydrolysis and protein aggregation, strongly influenced by factors such as rennet concentration, pH, and temperature, which directly affect gelation kinetics and curd firmness. Optical methods, such as those used in the Optigraph device, offer a non-invasive, real-time alternative to traditional rheological techniques.

This study aims to calibrate voltage signals from the Optigraph (generated by light transmission through milk samples) against measurements from a Haake Mars Rheometer using small amplitude oscillations. This calibration ensures voltage can reliably indicate curd firmness and key kinetic parameters.

Milk-clotting time (MCT) was determined according to ISO 23058/IDF 199:20061 with a slight modification in milk type. The milk substrate was characterized by pH measurement (Metrohm 713 pH meter, Herisau, Switzerland) and compositional analysis (fat, protein, lactose, total solids and solids-not-fat) using a MilkoScanTM Mars (Foss Electric, Hillerød, Denmark), according to ISO 21543:2020.

All analyses were performed in duplicate. Chymosin dilutions (1:40, 1:50, 1:60, 1:70) were tested in both systems to assess their effects on coagulation time, the R parameter (flocculation time) and MCT. [2]

The elastic modulus (G') serves as a direct measure of curd firmness and is the standard for monitoring gel development. Simultaneous measurements enabled the establishment of a quantitative relationship between voltage-time curves and G' evolution, allowing optical signals to be translated into rheological meaningful data, such as gelation time and firmness index.

The results showed a promising correlation between the Optigraph's R parameter and MCT. The optical detection of coagulation onset aligned closely with the initial rise in G'. [3]

This study validates the use of optical methods for real-time monitoring of milk coagulation and highlights their potential for automation in dairy processing. Future work will expand calibration across different milk types and coagulation conditions.

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Milk production and composition of pure Saanen and Saanen × Anglo-Nubian crossed goats at mid lactation

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The Saanen breed is one of the most widely used dairy goats in the world due to its high milk yields **Erro! A origem da referência não foi encontrada.**. Although less productive, the Anglo-Nubian breed is characterized by producing milk with high fat content.[0] As milk fat is of particular interest in cheese production, crossing these breeds may be of interest in dairy goat production. The aim of this study was to determine milk production and composition traits of purebred Saanen and Saanen × Anglo-Nubian crossbred goats throughout mid lactation.

Individual milk samples were collected from 60 lactating goats, distributed across four groups (n=15): 100% Saanen (S100), 75% Saanen / 25% Anglo-Nubian (S75AN25), 50% Saanen / 50% Anglo-Nubian (S50AN50), and 25% Saanen / 75% Anglo-Nubian (S25AN75). Each week, milk production was recorded in the morning and afternoon milking and samples were collected for milk protein (P), fat (F), lactose (L), total solids (TS), non-fat solids (NFS) using a mid-infrared spectroscopy instrument, MilkoScan™ Mars (Foss, Hillerød, Denmark) and pH analysis. As goats differed in stage of lactation, only data from the 6th to the 12th month of lactation were considered, as goats from all genotypes were simultaneously in production (S100=15, S75AN25=11, S50AN50=11, S25AN75=5). Daily data were analyzed using the MIXED procedure of SAS software, with the fixed effects of genotype, month of lactation and their interaction, the random effect of goat, and sampling week as repeated measures for each goat. Means were compared using Tukey's post-hoc test at the 5% significance level ($p<0.05$).

Daily milk yield was unaffected by genotype and month of lactation interaction ($p>0.05$). Genotypes S100 and S50AN50 showed higher milk production, followed by S75AN25, whereas S25AN75 exhibited the lowest yields ($p=0.026$). Although purebred Saanen were expected to have the highest milk yield, the equally high production yield of S50AN50 crossbred is a relevant and novel information. As expected, milk yield was observed to decrease as lactation progressed ($p<0.001$).

Milk composition (P, F, L, TS and NFS) was affected by the interaction between genotype and month of lactation ($p<0.05$). Regarding main milk constituents (P, F, L), pattern variations were found along lactation months (Fig. 1). Milk protein was the lowest in S50AN50 goats at 8th month of lactation and the highest in S25AN75 at months 7, 9, and 11 of lactation (Fig. 1). Compared to pure Saanen, milk protein was greater in S25AN75 goats but only in three of the six months analyzed (7, 8 and 11). Regarding milk fat, the lowest proportion was observed in S100 at 6th month of lactation and the highest in S75AN25 and S25AN75 at 12th and 6th month of lactation, respectively (Fig. 1). An increase in milk fat proportion would be expected in crossbred goats, but this was only observed at the 6th month of lactation when comparing S100 to S25AN75, and at 8th and 9th months of lactation for S75AN25 and S25AN75 genotypes comparing with pure Saanen. No effects were seen in fat proportion in later months among genotypes (Fig. 1). Lactose proportion was the lowest in S50AN50 in the 8th lactation month and the highest in S25AN75 in months 7, 8, 9, 10, and 11 and in S100 in the 12th month of lactation. However, withing each month, no differences in L were seen among pure and crossed goats, except in lactation month 8 (Fig 1). Milk TS were the highest in S25AN75 at the 7th, 8th, 9th, 10th and 12th lactation month and the lowest in S50AN50 at the 8th month. At the 8th month, S50AN50 also presented the lowest NFS, while the highest proportions were found in S100 and S25AN75 in all months and in S75AN25 and S50AN50 in months 6, 7, 9, 11 and 12. Milk pH was unaffected by all fixed effects ($p>0.05$).

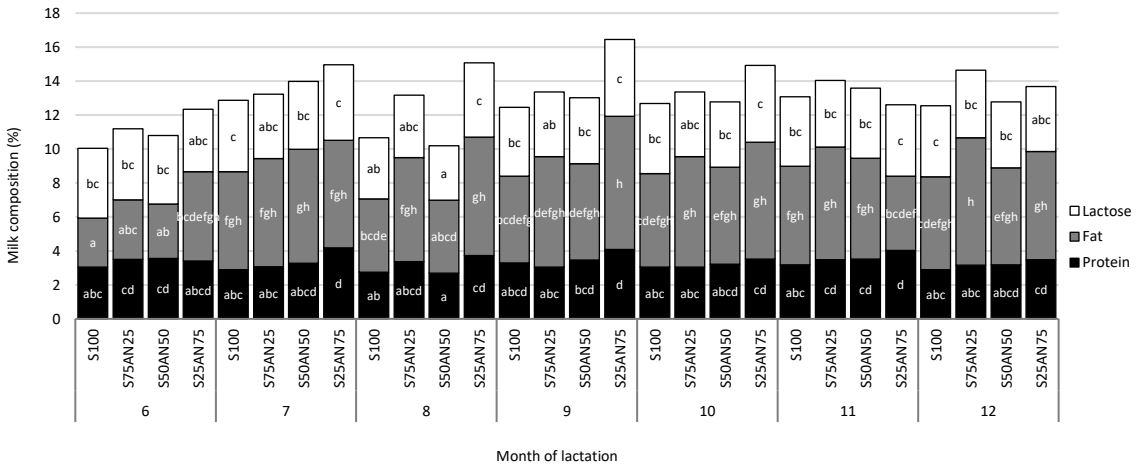


Figure 1 – Milk protein, fat, and lactose (%) throughout lactation in purebred Saanen (S100) and Saanen x Anglo-Nubian crossbred (S75AN25, S50AN50, and S25AN75) goats. a-h Bars with different superscripts are significantly different (p< 0.05)

Overall, results highlight the relevance of genetic background when aiming to improve milk yield and milk quality. The S25AN75 genotype produced lower milk yield but with the highest proportion of total solids in mid lactation, reflecting the highest protein and lactose in most lactation months. However, further studies should follow the entire lactation cycle and with higher number of animals to fully unveil its potential use in breeding programs focused on improving milk compositional quality in the scope of cheese production.

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[P.2.18]

The DAIRITAL Project: Promoting Sustainability and Digital Transformation in Traditional Dairy Production

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Traditional dairy farming and artisanal cheese production are essential activities for maintaining rural economies, preserving cultural heritage, and ensuring territorial cohesion across the SUDOE region (South-West Europe). However, the sector is currently facing significant challenges, including increasing production costs, climate-related pressures, limited access to technological innovation, and a lack of generational renewal. These factors threaten the long-term sustainability and competitiveness of dairy farms and small-scale cheese producers, many of which are microenterprises located in rural areas.

The DAIRITAL project (Development of Sustainable Production Systems in the Traditional Dairy Sector of the SUDOE Area) was established to address these common challenges through a multidisciplinary and transnational collaboration involving research institutions, producer associations, industry stakeholders, and governmental entities from Portugal, Spain, and France. The project seeks to promote the transition towards more sustainable, innovative, and digitally enabled dairy production systems, contributing to the resilience and competitiveness of the sector while supporting rural development and employment.

To achieve these goals, DAIRITAL combines experimental research, technological innovation, and pilot-scale validation in dairy farms and artisanal cheese production units. The project focuses on three major areas of intervention. The first concerns circular economy approaches, including the valorization and reuse of endogenous resources and by-products such as whey, wastewater, and livestock effluents, aiming to improve resource efficiency and reduce environmental impacts. The second area addresses agri-food technologies, evaluating how changes in production systems, herd management, and milking practices influence milk quality, technological properties, cheese sensory characteristics, and greenhouse gas emissions. The third area focuses on digital transformation through the adaptation and validation of rapid analytical tools, including mid-infrared spectroscopy (MIR), for predicting milk quality and cheese-making performance, as well as the development of digital decision-support systems for defect diagnosis and process optimization in dairy production.

The knowledge generated through experimental studies and real-scale pilot actions will support the development of a Transnational Strategy and a Transnational Action Plan aimed at facilitating the adoption of sustainable and digital solutions throughout the dairy value chain. Through training activities, knowledge transfer, and stakeholder engagement, DAIRITAL will contribute to improving the environmental, economic, and social sustainability of dairy production systems, strengthening the long-term viability of rural territories and enhancing the competitiveness of the traditional dairy sector across the SUDOE region.

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[P.2.19]

Advancing Antibiotic Residue Surveillance in Wastewater Using a Semi-Automated µSPEed–UHPLC–PDA Method

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The excessive use of antibiotics in livestock farming, driven by the demand for animal products, has raised concerns about residues entering the food chain and the aquatic environment.[1] Residues of commonly used antibiotics, such as sulfonamides, tetracyclines, and penicillins, can promote antibiotic resistance, alter gut microbiota, and cause allergic reactions or toxicity in consumers.[2] To address these risks, stricter regulations and robust analytical methods are required to detect low concentrations of these substances in complex environmental and agri-food matrices. Although conventional extraction techniques have been employed to isolate veterinary antibiotic residues prior to analysis, there is growing demand for more sustainable, miniaturised, and efficient extraction approaches in line with green chemistry principles.[3]

In this work, a semi-automated micro–solid-phase extraction (µSPEed) method coupled with UHPLC–PDA analysis was developed for the determination of six antibiotics — tetracycline, chlortetracycline, oxytetracycline, doxycycline, sulfamethoxazole, and trimethoprim — in wastewater samples related to livestock production. The extraction procedure was optimised by assessing 12 sorbent materials, including unmodified, modified, and functionalised silica, polymeric PS/DVB, and porous graphitic carbon sorbents. In addition, key operational parameters such as sample volume (500 and 800 µL), number of loading cycles (3–5), pH (2.5, 4.0, 7.0, and 8.5), and elution solvent (methanol, acetonitrile, and methanol containing 5% or 1% formic acid) were systematically evaluated. The best performance was achieved using a PS/DVB-RP sorbent with three 250 µL loading cycles and two successive elutions with 50 µL acidified methanol (0.1% formic acid). This configuration enabled rapid analysis with a total chromatographic run time of six minutes. Method validation demonstrated excellent linearity, precision, and recovery, with limits of detection between 0.30 and 1.23 µg L^{-1} and limits of quantification ranging from 0.92 to 3.73 µg L^{-1}. The µSPEed/UHPLC–PDA method proved to be an effective and environmentally conscious tool for monitoring antibiotic residues in wastewater, offering a reliable option to support food safety and environmental surveillance in livestock production systems.[3]

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[P.2.20]

Chemometric Fingerprinting of Wine Spirit Distillation Fractions by FT-Raman Spectroscopy

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Wine spirit is obtained through the distillation of wine, resulting in a distillate with a high ethanol content and a distinctive combination of volatile compounds. The quality of the final distillate is greatly affected by the variety of grapes used, the fermentation conditions and, in particular, the precise separation of the different distillation fractions, commonly known as 'distillation cuts'. For this study, wines were produced from Tália and Palomino Fino grapevine cultivars and distilled using two 20-litre Charentais copper pot stills. Two independent distillation replicates were performed for each wine. During each process, 125 mL fractions were collected sequentially, covering the entire run and including the head, heart and tail fractions. To evaluate the potential of rapid spectroscopic techniques for monitoring distillation cuts, all samples were analysed using FT-Raman spectroscopy (Bruker MultiRAM). Spectra were acquired with a spectral resolution of 8 cm^{-1} and a wavenumber range of 4000–200 cm^{-1}. The spectral data were subsequently processed using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) in order to evaluate the distribution and discrimination of the various distillation fractions. The results showed that FT-Raman spectroscopy combined with multivariate analysis can effectively distinguish between the main distillation fractions. PCA and PLS-DA enabled clear separation of the head, heart and tail samples, highlighting compositional differences throughout the distillation process. These results suggest that FT-Raman spectroscopy could offer distillers a quick, non-destructive method of identifying optimal cut points, thereby improving process control and spirit quality.

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XVIII Encontro de Química dos Alimentos

Coimbra | 14 - 16 de outubro'26

PROTEÍNAS ALTERNATIVAS, COMPOSTOS BIOATIVOS E ALIMENTOS HÍBRIDOS



Professora Catedrática da Faculdade de Biotecnologia da Universidade Católica Portuguesa, onde exerce também funções de Vice-Diretora e dirige o Centro de Biotecnologia e Química Fina (CBQF), Laboratório Associado. É Presidente do European Biotechnology Research Institute (EBRI), cofundadora do INSURE.hub e coordena o Laboratório de Investigação em Bioativos e Bioprodutos. Licenciada em Ciências Farmacêuticas pela Universidade do Porto e doutorada em Biotecnologia pela Universidade Católica Portuguesa, desenvolve investigação na área da biotecnologia aplicada ao desenvolvimento de ingredientes funcionais e bioativos, através da valorização de subprodutos e novos recursos. É autora de mais de 800 artigos científicos, inventora em 30 famílias de patentes e possui um h-index de 89. Tem coordenado diversos projetos nacionais e internacionais e desenvolvido colaborações com universidades, centros de investigação e empresas. Desde 2019, integra consistentemente o ranking dos 2% de cientistas mais citados do mundo na área da Agricultura, Pescas e Silvicultura.

Alternative proteins for the future of food: from sustainable resource valorisation to bioactive ingredients and food innovation

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The growing need to ensure food security while addressing health and environmental challenges is accelerating the transition towards more sustainable protein systems. Alternative proteins are increasingly recognised not only as nutritional resources but also as platforms for the generation of high-value functional ingredients capable of supporting the development of healthier and more sustainable foods.

This plenary lecture will present advances in the production of alternative protein ingredients from algae, insects, microbial biomass, and plant-based sources. A central focus will be the sustainable valorisation of food industry co-products and side streams from plant, animal and marine value chains as novel protein resources. Through circular bioeconomy approaches, these underutilised biomass streams can be transformed into high-value protein ingredients and bioactive fractions, contributing to increased resource efficiency, reduced waste generation, and the development of more sustainable food systems.

The role of green and scalable bioprocessing technologies will be discussed, highlighting enzymatic hydrolysis, membrane-based fractionation, electrolyte-assisted precipitation and fermentation as key tools to recover, concentrate and tailor protein functionality. These technologies enable the production of protein concentrates, hydrolysates and bioactive peptides with enhanced nutritional, technological and biological properties.

Special attention will be given to the assessment of protein quality through amino acid profiling, digestibility and bioaccessibility studies, as well as to the identification of bioactive peptides generated during processing. Recent findings illustrate how these ingredients can exert bioactive properties such as antioxidant, anti-inflammatory, antihypertensive, immunomodulatory and gut-health-promoting activities, opening new opportunities for the prevention of chronic diseases and the development of scientifically substantiated functional foods. Examples will be presented from research on protein hydrolysates and bioactive peptides obtained from alternative protein sources and from the upcycling of food industry by-products, demonstrating the potential of these resources to generate ingredients with both nutritional and health-promoting value.

Finally, the translation of these advances into food innovation will be explored through examples of functional foods, high-protein formulations, hybrid products and next-generation sustainable foods. By integrating biotechnology, nutrition, food engineering and industrial implementation, this presentation will showcase how alternative proteins can evolve from sustainable resources into scientifically validated bioactive ingredients, contributing to a resilient, circular and health-oriented food system.



Doutorada em Química pela Universidade de Aveiro, desde 2010, e Investigadora Auxiliar desde 2024. Integra, desde 2018, o LAQV-REQUIMTE, desenvolvendo a sua atividade no grupo de Bioquímica e Química Alimentar do Departamento de Química da Universidade de Aveiro. A sua investigação centra-se na Química e Bioquímica Alimentar, com especialização na química dos polissacarídeos, análise estrutural e valorização de subprodutos da indústria alimentar. Tem desenvolvido trabalho na caracterização estrutural de fibra dietética solúvel e no estudo da relação estrutura-função de polissacarídeos e oligossacarídeos, nomeadamente no desenvolvimento de alimentos com propriedades prebióticas e psicobióticas. Coordena e participa em diversos projetos de investigação nacionais e europeus, mantendo colaborações com várias indústrias alimentares.

The relevance of dietary fiber structure in precision fermentation: promotion of psychobiotic bacteria growth in the gut to enhance good mood

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Soluble polysaccharides and oligosaccharides are key dietary fiber components that can selectively modulate gut microbiota and promote the production of metabolites with potential effects on gut–brain communication. However, conventional fruit juice processing retains a substantial proportion of dietary fiber in the pulp, resulting in juices with relatively low levels of soluble fiber. This limitation can be addressed by enzymatic strategies designed to modify fiber structure and increase the release of soluble polysaccharides and oligosaccharides with potential prebiotic activity.

Apple cloudy juice contained approximately 4% dietary fiber (dry basis), predominantly composed of pectic polysaccharides. Enzymatic hydrolysis of apple pulp with endo- and exo-cellulases released 63.9% of the initial material, yielding monosaccharides together with cellulose- and xyloglucan-derived oligosaccharides. Treatment with pectin lyase, polygalacturonase, and pectin methylesterase released 57.7% of the material as pectic poly- and oligosaccharides. Fermentation with human fecal inocula increased the production of short-chain fatty acids (SCFAs), γ-aminobutyric acid (GABA), and 5-hydroxytryptophan, with pectinase treatment promoting changes in microbial composition and enhancing the production of metabolites involved in gut–brain communication. These results indicate that enzymatic modification of dietary fiber structure can influence its microbial utilization and metabolic outcomes.

More drastic strategies are required to recover soluble dietary fiber, potentially prebiotic polysaccharides from lignified nuts-processing by-products. Pine nut skin, a lignocellulosic by-product of pine nut processing, is a rich source of insoluble dietary fiber[1,2]. Subcritical-water extraction enabled the recovery of a soluble fraction enriched in pectic polysaccharides and xyloglucans[1]. Fermentation of this fraction produced changes in gut microbial composition comparable to those induced by fructooligosaccharides, used as a positive prebiotic control, including an increased abundance of *Bifidobacterium adolescentis*. The microbial community established during fermentation included several butyrate-producing taxa, concomitant with increased butyrate production. Importantly, microbial utilization was selective towards xyloglucans over rhamnose-containing pectic polysaccharides, demonstrating that polysaccharide structure and accessibility can determine substrate utilization by specific microbial populations[3]. Fermentation also resulted in the highest GABA concentration at 24 h.

Together, these findings highlight the relevance of dietary fiber molecular structure and accessibility in determining microbial fermentation patterns and metabolite production. Enzymatic modification of fruit-derived polysaccharides and the valorization of lignocellulosic by-products can therefore provide substrates for targeted modulation of gut microbial communities, including bacteria with psychobiotic potential. Such structure-guided approaches may contribute to the development of precision fermentation strategies aimed at enhancing the production of microbiota-derived metabolites involved in gut–brain communication and, ultimately, supporting mood and mental well-being.

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Nutritional Characterisation of Commercial Chlorella and Spirulina with focus on B-Group Vitamins, Amino Acids, Fatty Acids and Pigments

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Microalgae such as *Chlorella vulgaris (Chlorella) and Arthrospira platensis* (Spirulina) are increasingly recognized as sustainable sources of nutrients and bioactive compounds with potential applications in functional foods and nutraceuticals[1].

This study aimed to characterize the vitamin content of six commercial microalgae products (Figure 1) and to evaluate the nutritional composition of a representative sample (Spirulina nibs). Vitamin B12 was quantified in all samples, whereas vitamins B6 and B9 were analysed in selected Chlorella products. Vitamin B6 was determined by LC-FLD, vitamin B9 by an immunonephelometric assay, and vitamin B12 by HPLC-UV/DAD. Amino acid composition, fatty acid profile, and pigment content were investigated exclusively in the Spirulina sample using chromatographic and spectrophotometric methods.

Vitamin and aminoacid analyses were performed using samples taken from three independent batches of each commercial product (n = 3), and results are expressed as mean ± standard deviation (SD). For the fatty acids and pigment analyses, triplicate samples were taken from three independent batches of each product (n = 9), and results are expressed as mean ± standard deviation (SD).

Vitamin B12 concentrations ranged from 12.26 ± 5.11 µg/100g to 106.73 ± 42.02 µg/100 g, with the highest levels found in Chlorella, although further confirmation of bioactive forms is required. Vitamins B6 (1.08 ± 0.46 to 2.08 ± 0.30 mg/100 g) and B9 (929 ± 27 to 1066 ±1 27 µg/100 g) in Chlorella were present at nutritionally relevant levels. In Spirulina nibs, glutamic acid (6865 ±154 mg/100 g), aspartic acid (4540 ± 169 mg/100 g), alanine (3477 ± 110 mg/100 g), and leucine (3593 ± 81 mg/100 g) were the predominant amino acids, and all essential amino acids were detected, indicating high protein quality. Polyunsaturated fatty acids accounted for approximately 41% of total fatty acids, mainly linoleic (20.78% ± 0.37) and γ-linolenic acids (18.95% ± 0.52), highlighting the potential of Spirulina as a source of bioactive lipids. Chlorophyll a and carotenoid contents were 2.24 ± 0.30 mg/g and 0.69 ±0.12 mg/g, respectively.

Overall, these findings support the nutritional value of commercial *Chlorella* and Spirulina and their potential application in functional foods and human nutrition.



Figure 1 – Commercial Chlorella vulgaris (powder) and Spirulina (powder and nibs) samples under study.

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The role of Viola cornuta polyphenol-enriched extracts in the modulation of Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) involves epithelial barrier disruption and excessive inflammatory signaling, with oxidative stress playing a central role in disease progression. Polyphenol-rich plant extracts have emerged as promising modulators of intestinal oxidative and inflammatory stress, and *Viola cornuta*, an edible flower, contains bioactive compounds with reported antioxidant and anti-inflammatory properties. Four color-based variants of *V. cornuta* (**Figure 1**), differ markedly in phenolic composition: DP and MP are characterized by a predominance of anthocyanins, whereas Y and LB present a more diverse profile of other



Figure 1 - Four color-based variants of V. cornuta — Light Blue (LB), Mid Purple (MP), Deep Purple (DP), Yellow (Y) – respectively.

polyphenols with comparatively lower anthocyanin content. Clarifying how this compositional variability relates to the antioxidant and anti-inflammatory potential of each variant, and consequently to their capacity to modulate IBD-associated outcomes, is a key aim of this work. This study evaluated the safety of different V. cornuta extracts and their protective effects against inflammation-induced epithelial stress using in vitro intestinal models. Cytotoxicity of four V. cornuta extracts (12.5–200 µg/mL) was assessed in Caco-2 and HT-29 cells using the MTT assay to determine non-toxic concentration ranges. Lipopolysaccharide (LPS) assays were then performed to identify stress-inducing conditions without major viability loss, with reactive oxygen species (ROS) production confirming effective stress induction. To better mimic the intestinal microenvironment, a transwell co-culture system combining Caco-2 and HT-29 cells was established, and cells were pre-treated with selected extract concentrations before LPS exposure to evaluate protective effects. To further characterize the variant-specific immunomodulatory potential of V. cornuta, gene expression of the pro- and anti-inflammatory markers IL-10, IL-6, TNF-α and IL-1β was assessed by qPCR in Caco-2 and HT-29 cells cultured separately, following 24 h incubation with each of the four color variants (50 µg/mL) and subsequent stimulation with LPS (500 µg/mL). All extracts showed low cytotoxicity in both cell lines, with IC_{50} values above 200 µg/mL; cell viability remained above 80% up to 100 µg/mL and above 50% at 200 µg/mL, supporting the selection of safe working concentrations. In LPS-stimulated monocultures, LPS increased intracellular ROS levels, confirming effective stress induction, while pre-treatment with selected V. cornuta extracts reduced ROS production and restored cell viability close to basal levels. In the transwell co-culture model, LPS exposure compromised epithelial barrier integrity, whereas extract pre-treatment preserved barrier function, maintained higher viability, and reduced stress-related alterations. Overall, V. cornuta extracts demonstrated a favorable safety profile and effectively counteracted LPS-induced oxidative stress. Reduced ROS production, preserved cell viability, and maintenance of epithelial barrier integrity support their protective role against intestinal epithelial dysfunction, while the variant-specific modulation of IL-10, IL-6, TNF-α and IL-1β expression is expected to further clarify the anti-inflammatory mechanisms underlying each V. cornuta color variant. These findings support the hypothesis that anthocyanin-rich variants (DP, MP) and variants richer in other polyphenols (Y, LB) may act through distinct or complementary pathways, highlighting the anthocyanin/polyphenol content of each V. cornuta color variant as a key determinant of its bioactive potential and justifying further mechanistic and functional studies in the context of inflammatory bowel disease.

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Giving Wine Lees a Second Life: Bioactive Effect's on Probiotics Performance and Intestinal Inflammation

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Traditionally regarded as a low-value winery by-product, wine lees are increasingly being recognised as a rich reservoir of bioactive compounds with potential applications far beyond their nutritional value. Rich in dietary fibre, yeast-derived polysaccharides, phenolic compounds, and other functional molecules, wine lees may contribute to gut health by supporting probiotic performance and influencing host–microbe interactions. As interest grows in the development of sustainable and microbiota-targeted food ingredients, this work evaluated the potential of extracts obtained from white, red, and Port wine lees to modulate probiotic growth, adhesion, and immunomodulatory activity.

To explore the potential of wine lees as gut health-promoting functional ingredients, extracts obtained from white, red, and Port wine lees were first screened for cytocompatibility towards Caco-2 intestinal epithelial cells. Following confirmation of their safety, the extracts were evaluated for their capacity to support the growth of Lacticaseibacillus casei 01 and Lactiplantibacillus plantarum 299v as alternative substrates for probiotic metabolism. Bacterial growth was assessed under distinct nutritional conditions, including both glucose-rich and glucose-limited environments, and monitored over time through viable cell enumeration. Given the central role of probiotic colonisation in mediating host–microbe interactions, the influence of wine lees extracts on bacterial adhesion was subsequently evaluated using intestinal adhesion models. Beyond their effects on probiotic performance, the functional relevance of these interactions was further explored in an in vitro intestinal inflammation model. The immunomodulatory potential of the combined presence of probiotics and wine lees extracts was assessed following inflammatory stimulation through the quantification of the pro-inflammatory cytokines IL-6 and TNF-α.

The results demonstrated that both probiotic strains were capable of growing in the presence of wine lees extracts, even under glucose-limited conditions, indicating that components present in the extracts can serve as alternative substrates for bacterial metabolism. These findings highlight the potential of wine lees-derived compounds to support probiotic growth and persistence. Furthermore, the extracts had no negative effects on probiotic adhesion to intestinal cells, regardless of the strain and the type of wine lees extract considered. From an immunomodulation standpoint distinct responses were observed among the different extracts and probiotic combinations, indicating that wine lees-derived compounds may influence host inflammatory pathways through mechanisms involving both direct interactions with intestinal cells and probiotic-mediated effects.

Overall, wine lees extracts supported probiotic growth without compromising adhesion capacity and modulated intestinal inflammatory responses, highlighting their potential as sustainable functional ingredients for gut health applications. These findings further demonstrate how winery side streams can be transformed into high-value bioactive ingredients within a circular food system.

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Linking volatile chemistry with sensory perception for informed selection of microalgae for targeted food applications

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Microalgae are increasingly recognized as sustainable food ingredients with remarkable nutritional and functional potential. Nevertheless, their successful incorporation into food products remains limited by technological challenges, production costs and, above all, their characteristic sensory profile, which often compromises consumer acceptance[1]. Current formulation strategies largely rely on empirical approaches, where microalgal biomass is incorporated into food matrices and only subsequently evaluated for sensory acceptance. Such trial-and-error approaches limit the rational development of microalgae-based foods and overlook the intrinsic chemical diversity among microalgal biomasses.

Rather than asking whether microalgae can be incorporated into foods, a more relevant question is which microalgal biomass is best suited for a given food application. Addressing this challenge requires a deeper understanding of the relationship between biomass characteristics and their technological and sensory performance. Aroma is one of the primary drivers of consumer acceptance, and volatile organic compounds (VOCs) largely define the sensory identity of microalgae. Since VOC composition is strongly influenced by biomass characteristics, cultivation conditions and downstream processing, volatile fingerprints provide valuable chemical information to support a more informed selection of microalgal ingredients according to their intended food application[2]. The proposed framework is illustrated in Figure 1, highlighting how biomass characteristics, volatile chemistry and sensory perception can support informed ingredient selection. However, these relationships remain poorly explored, emphasizing the need to move beyond empirical formulation towards a more rational selection of microalgal ingredients.

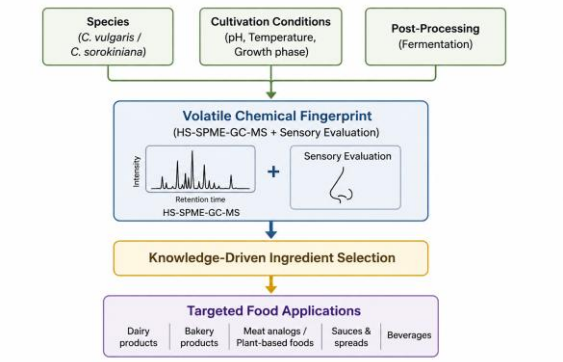


Figure 1 - Conceptual framework for knowledge-Driven selection of microalgal ingredients.

To address this challenge, the present work was developed within the Algaelicious PT2030 project promoted by Allmicroalgae Natural Products, providing access to heterotrophically cultivated Chlorella biomasses produced under controlled industrial conditions. As an initial proof of concept, this study investigates the effect of downstream fermentation on the volatile and sensory profiles of heterotrophically cultivated Chlorella biomasses.

Volatile compounds were characterized by headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME-GC-MS). To facilitate interpretation of volatile fingerprints in relation to aroma perception, a preliminary descriptive sensory evaluation was conducted by a semi-trained panel experienced in microalgal research. Panelists independently evaluated the aroma of identically prepared samples after 30 min headspace equilibration at 60 °C, matching the conditions used for HS-SPME-GC-MS analysis and enabling direct comparison between volatile fingerprints and their corresponding aroma descriptors within each biomass.

Preliminary results demonstrated that the proposed workflow successfully discriminated microalgal biomasses according to their volatile composition. As an initial proof of concept, downstream fermentation of heterotrophically cultivated Chlorella vulgaris (mutant, Honey) induced a marked restructuring of the volatile fingerprint, characterized by a substantial increase in acetic acid, a pronounced reduction in 2,3-butanediol and other alcohols, and the appearance of ester- and furan-related compounds, consistent with fermentation-driven metabolic transformations. These compositional changes were accompanied by a shift in aroma perception from predominantly green and mushroom-like notes towards more acidic and fermented descriptors, demonstrating the close relationship between volatile composition and sensory perception.

Overall, these findings support the concept that volatile fingerprints can serve as chemical signatures to guide the informed selection of microalgal biomasses according to their aroma potential. As additional datasets encompassing different Chlorella biomasses, cultivation conditions and processing strategies become available, this approach is expected to contribute to a robust framework for selecting the most suitable microalgal ingredients for targeted food applications, ultimately reducing empirical formulation and supporting the development of products with improved sensory acceptance.

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Exploring Aromatic Plant Phenolics as Natural Modulators of Adipocyte Metabolism

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Obesity is no longer viewed solely as a consequence of excessive fat accumulation but as a complex metabolic disorder characterized by adipose tissue dysfunction and altered endocrine activity, both of which contribute to the development of metabolic diseases. In this context, phenolic compounds from aromatic and medicinal plants have attracted considerable interest due to their ability to modulate metabolic pathways associated with adipocyte function[1,2]. This study investigated the effects of phenolic extracts obtained from Salvia rosmarinus, Aloysia citrodora, Cymbopogon citratus, and Thymus vulgaris on adipocyte function, focusing on adipogenesis, lipolysis, and adipokine secretion.

Extracts fingerprinting through high resolution LC-MS showed that between 24 and 48 individual compounds were identified, with the highest content being observed for S. rosmarinus and phenolic classes representing between 58 and 63% of the total metabolites extracted. Next differentiated 3T3-L1 adipocytes were treated with the characterized extracts, and their impact on lipid metabolism and endocrine activity was assessed. The results demonstrated that all extracts (without major differences) significantly enhanced lipolytic activity, increasing glycerol release by ca.20% and thus promoting the mobilization of intracellular lipid stores. In parallel, the extracts effectively modulated adipokines metabolism and for both S. rosmarinus presented the highest activity. Leptin was, on average, reduced by 16% by all extracts, suggesting a beneficial effect on adipocyte endocrine regulation. On the other hand, a strong effect upon adiponectin secretion was observed for all extracts with increases of 20-50%, depending on the plant source, indicating an improvement in adipocyte metabolic function. The extracts also influenced adipogenesis, reducing it on average by ca. 15%, highlighting their ability to modulate adipocyte differentiation and lipid accumulation.

Overall, these findings suggest that phenolic extracts from aromatic and medicinal plants may modulate adipocyte function by stimulating lipolysis and improving adipokine secretion profiles under the in vitro conditions evaluated. By influencing multiple aspects of adipocyte metabolism, these extracts show potential as functional ingredients for nutraceutical strategies targeting obesity and associated metabolic disorders. However, further validation in more physiologically relevant models and in vivo studies is required to confirm their efficacy and translational potential.

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From INFOGEST Digestion to Potential Colonic Functionality: Structure–Function Relationships of Upcycled Intrinsic Antioxidant Dietary Fibre Ingredients

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Agro-food by-products are promising sources of bioactive, upcycled fibre-rich ingredients. While many studies focus on isolated fibres, particularly soluble dietary fibre (SDF) due to its high fermentability, less attention has been given to insoluble dietary fibre (IDF) and to intrinsic fibre preserved within plant tissues.[1] In plant foods, fibres form a three-dimensional cell wall network of cellulose, hemicelluloses and pectin, which can entrap starch, proteins, soluble sugars and lipids, while maintaining fibre-bound bioactives, including phenolic compounds.[1] Preserving this organisation may provide functionality that differs from purified fibres, by modulating bioaccessible release during digestion and the properties of the fibre-rich material available for colonic fermentation.[2]

This study aimed to understand how minimally processed, upcycled intrinsic antioxidant dietary fibre (IADF) ingredients are transformed during gastrointestinal digestion and how their structural features influence the release or retention of bioactive compounds. Particular attention was given to matrix composition, physical structure and particle organisation as determinants of bioaccessibility and the bioactive potential of the fibre-rich material reaching the colon.

Six plant-based by-products — brewer's spent grain, olive pomace, grapevine pruning, almond hull, apple pomace and tomato pomace — were evaluated as sources of upcycled IADF. Before digestion, the matrices were characterised for proximate composition, including dietary fibre fractions, cell wall monosaccharides, water retention capacity, solubility, particle size distribution and phenolic-associated antioxidant potential. Total dietary fibre ranged from 48.0% in almond hull to 82.1% in grapevine pruning, with IDF as the major fraction in all samples (38.8–79.0%). SDF varied from 3.0–5.4% in brewer's spent grain, grapevine pruning and olive pomace to 10.1–11.9% in tomato and apple pomace. Protein ranged from 4.3% in almond hull to 23.9% in brewer's spent grain, lipids from <1% in almond hull and grapevine pruning to 10.2–11.5% in brewer's spent grain and tomato pomace, and soluble sugars from 1.1% in apple pomace to 26.0% in almond hull. Water retention capacity ranged from 12.0 to 20.1 g/g, and water solubility index from 6.7% to 36.8%. Olive pomace presented the highest proportion particles below 50 µm, while olive pomace, grapevine pruning and almond hull showed high phenolic-associated antioxidant potential, including considerable content in bound phenolics.

The standardised INFOGEST in vitro digestion protocol was applied to assess the digestive fate of these ingredients. Bioaccessible fractions and post-digestion fibre-rich fraction were recovered separately. The bioaccessible fraction was analysed for soluble dry matter, total phenolic compounds, antioxidant capacity and phenolic profiles by LC-MS. The post-digestion fibre-rich pellet was characterised as material potentially available for colonic fermentation. Recovery yield indicated matrix resistance to upper gastrointestinal digestion, while monosaccharide composition, bound phenolics and antioxidant capacity provided information on structural and bioactive components remaining associated with the fibre matrix. FTIR and SEM analyses before and after INFOGEST followed chemical and microstructural changes, linking matrix disruption and compound release with fibre-matrix integrity.[3]

After INFOGEST digestion, clear differences were observed in the distribution of soluble material, antioxidant capacity and phenolic compounds between the bioaccessible fraction and the post-digestion fibre-rich fraction. These results showed that matrix organisation affects both bioactive release during digestion and the preservation of fibre-associated functionality in the material reaching the colon.

Overall, this study demonstrates that the functionality of upcycled IADF ingredients is determined not only by composition, but also by the structural organisation of fibre, bioactive compounds and other matrix components. By linking INFOGEST bioaccessibility data with the structural and physicochemical properties of the post-digestion fibre-rich fraction, the results reveal that intrinsic plant cell wall organisation governs the balance between bioactive release during digestion and fibre-associated bioactivity in the material reaching the colon. This work highlights the underexplored potential of minimally processed, fibre-rich plant matrices and supports the design of functional and prebiotic ingredients for sustainable and circular food systems.

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High-Moisture Extrusion of Pea Protein and Dulse (Palmaria palmata) Seaweed: Effects of Thermal Processing and Mechanical Shear on Protein and Mineral Bioaccessibility

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Extruded products represent an emerging food category by incorporating non-conventional processing methodologies, expanding consumption opportunities and challenging conventional boundaries within contemporary food systems. The technology to perform food extrusion has a wide range of applications, particularly in the production of high-moisture[1] or low-moisture extrudates, using diverse raw materials. High-moisture extrusion (HME) is a promising technology for producing plant-based meat analogues with desirable texture properties while enabling the use of diverse and sustainable raw materials. Extrusion processing can preserve essential nutrients, improve their bioaccessibility, increase soluble dietary fiber and reduce antinutritional factors, thereby contributing to the development of nutritious and sustainable foods[2]. However, the combined effects of HME and seaweed incorporation on protein digestibility and mineral bioaccessibility remain poorly understood.

HME was performed using a Process 11 Parallel Twin-Screw Extruder (Thermo Scientific, Germany). Screw speed, liquid feed rate, powder feed rate, barrel temperature and die temperature were optimized to produce meat analogues with the desired textural properties, using a maximum barrel temperature of 140°C. HME was applied to pea protein-based formulations containing 10% and 15% Palmaria palmata (dulse). In addition to texture profile analysis, in vitro digestion was performed according to the INFOGEST 2.0 protocol, followed by the determination of protein digestibility and mineral bioaccessibility. These results were compared with formulations prepared using the same powder-to-liquid ratio and baked in a conventional oven at 140°C.

This comparison enabled the differentiation of the effects associated with extrusion from those resulting solely from thermal processing, since both products underwent heat treatment but differed substantially in processing conditions. While baking involves prolonged heating with minimal mechanical shear, HME combines high temperature, intense shear, and short residence time, resulting in distinct structural modifications. This experimental design allows the differentiation of the effects of thermal treatment and mechanical shear on protein digestibility and mineral bioaccessibility. The structural modifications induced by extrusion are expected to promote the formation of fibrous, meat-like structures through protein alignment and crosslinking during processing[3].

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From microalgae volatile profiles to wheat bread aroma: linking VOCs with sensory perception

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Microalgae are attracting increasing interest as sustainable food ingredients due to their high nutritional value and functional potential.[1] However, their characteristic aroma, often described as marine, fishy, grassy, and earthy, remains a major barrier to consumer acceptance [2]. These sensory properties are largely driven by volatile organic compounds (VOCs), which vary among species and can be modified by downstream processing. This study investigated the volatile composition and sensory characteristics of *Chlorella vulgaris*, *Microchloropsis gaditana,* and *Limnospira platensis* (Spirulina) and evaluated how their volatile profiles are translated into sensory perception after incorporation into wheat bread. VOCs were characterized by static headspace gas chromatography–mass spectrometry (SHS–GC–MS). Sensory profiling of the microalgae biomasses was performed using the Check-All-That-Apply (CATA) methodology. Wheat breads containing 4% microalgae were subsequently evaluated using the Rate-All-That-Apply (RATA) methodology. A total of 40 VOCs were identified across all species, with aldehydes, ketones, N-based compounds, and alcohols representing the predominant VOC classes. Multiple Factor Analysis (MFA) clearly discriminated the three species according to their volatile and sensory profiles. *M. gaditana* was associated with fish-related odours and aldehyde-rich volatile profiles, whereas *C. vulgaris* showed marine and herbal notes and *Spirulina* exhibited floral and mineral characteristics. RATA profiling indicated that the odour characteristics identified by CATA were only partially retained after incorporation into wheat bread. These results demonstrate that volatile signatures identified in isolated biomasses are not directly translated into the final food product. Overall, both formulations exhibited moderate marine-related notes and low intensities of undesirable attributes, such as rancidity. *C. vulgaris* bread exhibited more pronounced sea air and fish-related odour notes, whereas Spirulina bread was characterised by a less pronounced marine odour profile. Despite these sensory differences, both formulations achieved consumer acceptance scores above 6 on a 9-point hedonic scale.

These findings demonstrate that volatile signatures identified in isolated microalgae are substantially modified after food processing, highlighting the importance of food matrix interactions in shaping aroma perception. This knowledge supports the rational selection of microalgae for specific food applications while contributing to the development of products with improved sensory quality.

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Antibacterial and Anticancer Potential of Apple Pomace and Its Application in Functional Food Products

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Apple pomace is one of the major by-products of the fruit juice industry, accounting for approximately 25–30% of the original apple mass after juice extraction. It is commonly discarded as food waste or used as animal feed despite being a rich source of dietary fiber and bioactive compounds with considerable potential for the development of value-added functional foods.[1] The aim of this study was to investigate the antibacterial and anticancer activities of apple pomace and to evaluate the influence of particle size on these bioactivities. In addition, the antibacterial activity of apple pomace was assessed after its incorporation into crackers and smoothies to evaluate its potential as a functional food ingredient and a natural clean-label preservative.

Antibacterial activity against *Escherichia coli* O157:H7 was evaluated using aqueous extracts (6, 3, and 1.5 mg/mL) prepared from apple pomace fractions with different particle sizes (A: whole pomace; B: particle size ≥ 1 mm; C: 1 mm > particle size ≥ 0.71 mm; D: 0.71 mm > particle size ≥ 0.18 mm; and E: 0.18 mm > particle size ≥ 0.075 mm) using the broth microdilution method. The antibacterial activity of crackers containing 0, 20, and 40% apple pomace against *E. coli* O157:H7 was assessed using aqueous extracts (6 mg/mL) by the broth microdilution method, whereas smoothies containing 0, 5, and 10% apple pomace were evaluated using the spread plate method. The anticancer activity of selected apple pomace fractions (A, B, and D) against HT29 colorectal cancer cells was assessed using a wound healing assay.

The results showed that aqueous extracts of all apple pomace fractions exhibited inhibitory activity against *E. coli* O157:H7 at all tested concentrations. The highest inhibition (43.41%) was observed for fraction B (particle size ≥ 1 mm) at 6 mg/mL. Crackers containing 20 and 40% apple pomace exhibited inhibitory activities of 58.47 and 61.20% against *E. coli* O157:H7, respectively, with no significant difference between the two formulations ($p > 0.05$). Smoothies containing 5 and 10% apple pomace showed inhibitory activities of 94.5 and 100%, respectively, against *E. coli* O157:H7 (**Figure 1**). Regarding anticancer activity, aqueous extracts (500 mg/mL) of freeze-dried *invitro*-digested apple pomace reduced the migration of HT29 colorectal cancer cells by 8.52%, 42.35% and 20.62% for fractions A, B and D, respectively, after 48 h of incubation compared with the control.

These results show a huge potential for the use of dried apple pomace as an ingredient in staple foods, to increase food safety and with potential anti-cancer impacts on health.

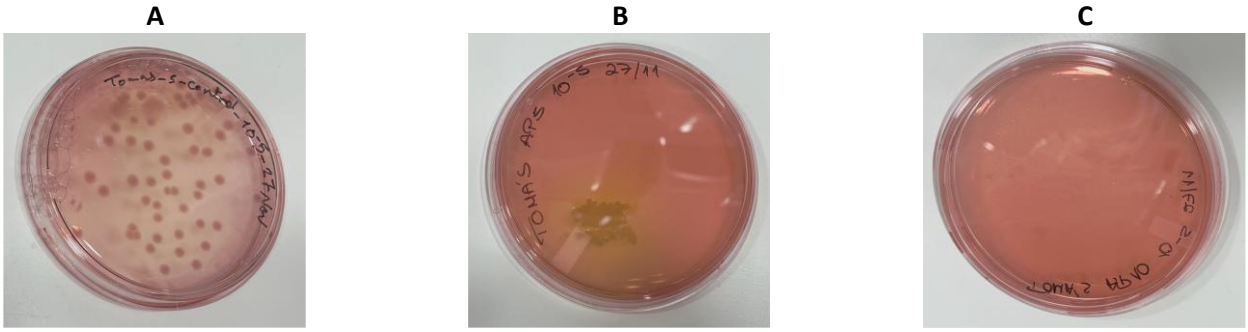


Figure 1 - Agar plates showing Escherichia coli O157:H7 count after inoculation in smoothies with different levels of apple pomace: (A) Control smoothie (0% apple pomace), (B) smoothie containing 5% apple pomace, and (C) smoothie containing 10% apple pomace.

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Towards Hypoallergenic Lupine-Based Ingredients by Enzymatic Hydrolysis

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White lupine (Lupinus albus) is a nutrient-dense legume widely used as a high-protein, high-fibre and low-carbohydrate ingredient in gluten-free and ketogenic formulations. However, its wide application is limited by its allergenic potential, thus representing an increased health risk for sensitised/allergic individuals. This study evaluated whether autoclaving combined with enzymatic hydrolysis could reduce the IgG/IgE-binding capacity of lupine proteins, with the aim of developing a hypoallergenic ingredient suitable for sports nutrition and gluten-free foods. For this purpose, autoclaved and non-autoclaved lupine flours were subjected to hydrolysis with commercial proteases, such as Alcalase, Neutrase, and papain, at different concentrations. Protein profiles were analysed by SDS-PAGE, while IgG/IgE-binding capacity was assessed via immunoblotting and ELISA using anti-γ-conglutin antibodies and sera from lupine-allergic patients. The degree of hydrolysis was assessed using the OPA-NAC protocol.

A mathematical model was firstly applied to determine the optimal hydrolysis conditions, which were established only for Alcalase and subsequently used to produce a dried hypoallergenic lupine protein hydrolysate. This hydrolysate was later characterized by high-resolution mass spectrometry (HR-MS) and incorporated into two high-protein food products (pizza and hamburger) at different percentages. Alcalase combined with pre-autoclaving resulted in the highest degree of hydrolysis (~40%) and the greatest reduction in IgG-binding (87.8%) compared with the control and the Neutrase- and papain-treated samples. The model predicted that the optimal hydrolysis conditions were 60°C for 30 minutes at an enzyme concentration of 0.5% (v/v). HR-MS analysis revealed that the resulting peptides were primarily derived from γ-conglutin, β-conglutin (isoforms 2 and 5), and cupin proteins, with a median peptide length of five amino acids (interquartile range: 2–7 residues). Food products formulated with the hypoallergenic lupine hydrolysate exhibited a substantial reduction in protein/peptide IgE-reactivity when tested with sera from lupine-allergic individuals. These findings support the potential of enzymatic hydrolysis as a strategy to reduce lupine protein allergenicity and facilitate the development of hypoallergenic lupine-based ingredients for functional foods.

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Microalgae-enriched 3D-printed cereal-based snacks: A strategy for personalized nutrition through texture design and nutritional enhancement

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Three-dimensional (3D) food printing is emerging as an innovative technology for producing personalised foods with tailored nutritional profiles and textural properties while enabling the incorporation of sustainable functional ingredients. Among these, microalgae represent a promising alternative due to their high protein, mineral and bioactive compound contents[1,2]. However, few studies[2,3] are evaluating their effects on the printability, technological performance and nutritional quality of cereal-based 3-D-printed foods, particularly those enriched with *Microchloropsis gaditana*.

This study aimed to develop cereal-based 3D-printed snacks enriched with Spirulina (*Limnospira platensis*) and *M. gaditana* (4, 6 and 10%, w/w) and to evaluate their rheological, technological, physicochemical, nutritional, bioactive and sensory properties. All dough formulations exhibited shear-thinning behaviour and dominant elastic characteristics (G' > G"), confirming their suitability for extrusion-based 3D printing. Increasing microalgae incorporation strengthened the dough structure, maintaining excellent printability and shape fidelity after printing and baking. Although microalgae significantly modified dough and snack colour, no adverse effects were observed on product dimensions or hardness. Water activity remained below 0.32 in all baked snacks, indicating favourable conditions for microbial stability and maintenance of crispy texture. Microalgae enrichment substantially improved the nutritional value of the snacks. Protein content increased from 8.07% in the control to 15.41% in snacks containing 10% Spirulina and to 13.10% in those containing 10% *M. gaditana* snack, corresponding to increases of approximately 91% and 62%, respectively. Ash and essential mineral contents also increased significantly, enabling several formulations to meet the requirements for the European nutrition claim "source of protein", while all enriched snacks qualified as "high in iron". Vitamin B12 content was also detected in the microalgae-enriched formulations, further enhancing their nutritional potential. Furthermore, antioxidant capacity and total phenolic content increased with algal biomass concentration, with *M. gaditana* formulations exhibiting the greatest bioactive potential.

Sensory evaluation demonstrated that snacks containing 4% Spirulina achieved the highest overall consumer acceptance and purchase intention, indicating that moderate biomass incorporation achieved the best balance between nutritional enhancement and consumer acceptance.

Overall, this study demonstrates that both Spirulina and *M. gaditana* are promising functional ingredients for extrusion-based 3D food printing, enabling the production of technologically stable snacks with enhanced nutritional quality, tailored textural properties and high consumer acceptance. These findings demonstrate how 3D food printing can integrate sustainable functional ingredients with customised nutritional composition and texture, paving the way for next-generation foods designed to meet individual nutritional needs and consumer preferences.

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Pterospartum tridentatum (Carqueja) Flower Extract: A Natural Strategy to Protect Sunflower Oil

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Edible oils are important components of the human diet and are widely used in food preparation, particularly for deep-frying. However, prolonged exposure to oxygen, high temperatures, and moisture promotes oxidative and hydrolytic reactions, leading to progressive oil deterioration. During oxidation, hydroperoxides are initially formed and subsequently decompose into secondary products, including aldehydes, ketones, and alcohols. In parallel, hydrolysis breaks down triacylglycerols, releasing partial acylglycerols and free fatty acids. The accumulation of these degradation products contributes to rancidity, nutritional losses, undesirable sensory changes, and the formation of potentially harmful compounds. Conventional sunflower oil is particularly susceptible to oxidative deterioration because of its high content of polyunsaturated fatty acids, especially linoleic acid.[1]

Antioxidants can retard lipid oxidation by inhibiting free-radical chain reactions and reducing the formation of primary and secondary oxidation products. However, the limited thermal stability of some synthetic antioxidants, together with the increasing demand for natural and sustainable food ingredients, has stimulated the search for plant-derived alternatives. One potential source is *Pterospartum tridentatum*, commonly known as carqueja, a plant that grows wild in Portugal and has long been used in traditional medicine and regional cuisine. Its flowers contain a diverse range of phenolic compounds, particularly flavonoid and isoflavonoid derivatives, supporting the potential use of flower-derived extracts in food applications.[2]

The present study assessed the ability of an ethanolic extract from *P. tridentatum* flowers to improve the storage and thermal stability of refined sunflower oil.[3] The extract exhibited DPPH radical-scavenging activity, with an IC_{50} of 76.3 ± 2.6 µg/mL, and a total phenolic content of 200 ± 8 mg GAE/g. It was incorporated into sunflower oil at concentrations of 500 and 1000 mg/L, while unsupplemented oil served as the control. Oil stability was evaluated during 30 days of storage at room temperature, with sampling at 0, 15, and 30 days, and during 27 h of heating at 180 °C to simulate deep-frying conditions, with samples collected every 3 h. Deterioration was monitored through acid value (AV), peroxide value (PV), p-anisidine value (p-AV), total oxidation value (TOTOX = 2 × PV + p-AV), and the UV spectroscopic determination of conjugated dienes (CD), and conjugated trienes (CT), using standardized methods. Refractive index (nD^{20}) was measured using an Abbe refractometer, while density was determined at room temperature using a hydrometer with a measurement range of 0.900–1.000 g/mL. Experiments were performed in triplicate, and results are expressed as mean ± SD. Statistical analyses were conducted in SPSS 25 using one-way ANOVA followed by Tukey's test, or the Kruskal–Wallis test when ANOVA assumptions were not met ($P \leq 0.05$). PCA was performed in CANOCO 4.5 using log-transformed data.

After 30 days of storage, the highest extract concentration provided the greatest protective effect. Compared with the control, it reduced AV by 22.4%, PV by 17.2%, and p-AV by 45.6%, indicating lower hydrolytic deterioration and reduced formation of primary and secondary oxidation products. This protective effect was maintained under simulated deep-frying conditions. After 27 h at 180 °C, oil supplemented with 1000 mg/L extract exhibited significantly lower AV (0.216 ± 0.016 mg KOH/g) and TOTOX (69.30 ± 0.26) than the control. PCA of the heating assay confirmed the protective effect of the extract (Figure 1). Control samples showed continuous deterioration throughout heating, with the poorest quality parameters after 27 h. Supplemented oils heated for up to 18 h showed lower acidity and oxidative parameters than those heated for 21–27 h, indicating that secondary oxidation became more expressive only after 21 h. Oil quality improved with increasing extract concentration (control < 500 mg/L < 1000 mg/L), with the 1000 mg/L treatment showing the greatest stability.

Overall, these findings demonstrate that P. tridentatum flower extract delayed the hydrolytic and oxidative deterioration of sunflower oil during storage and prolonged heating. Its phenolic content, antioxidant activity, and thermal effectiveness support its potential as a sustainable natural ingredient for extending the shelf life and improving the stability of edible oils.

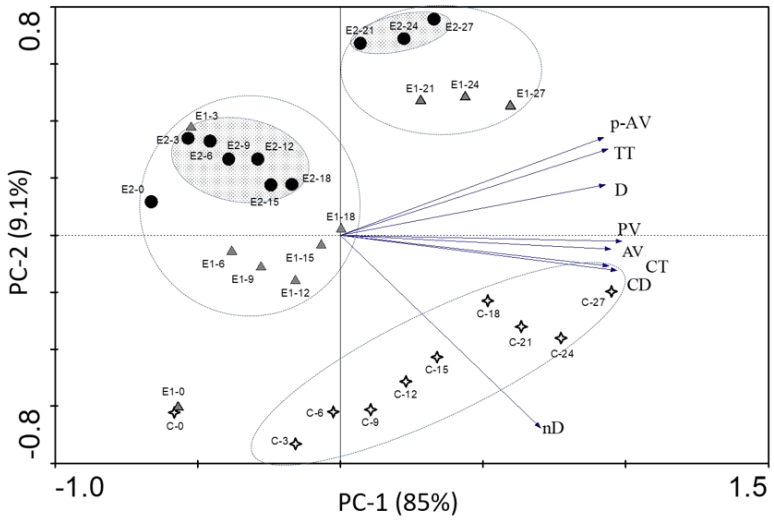


Figure 1 - PCA of sunflower oil quality parameters along 27 h of heating at 180 °C. PC1 and PC2 explained 85.0% and 9.1% of total variability. C, E1, and E2 represent control oil and oil supplemented with 500 and 1000 mg/L extract, respectively.

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Nutritional and functional characterization of sourdough bread enriched with pea protein

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Over the past few decades, the growing prevalence increasing of chronic non-communicable diseases has been associated with unhealthy lifestyles and increased consumption of ultra-processed foods.[2,1] In this context, the development of products that promote a balanced diet and are enriched with functional ingredients has become a major trend in the food sector, with bread emerging as one of the most promising vehicles due to its wide acceptance and versatility.[1] Among sustainable plant-based protein sources, pea (Pisum sativum L.) stands out for its potential to fortify cereal-based products, enhance their nutritional quality, and promote the release of bioactive compounds.[3] Thus, the present study aimed to develop an innovative bread enriched with textured pea protein and naturally fermented with sourdough, and to evaluate the effects of this formulation on the nutritional, technological, and functional properties of the final product. The nutritional characterization was performed according to official AOAC methods. The total and free amino acid profiles were determined by ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-Orbitrap MS), while the fatty acid and free sugar profiles were analyzed by gas chromatography with flame ionization detection (GC-FID) and high-performance liquid chromatography with refractive index detection (HPLC-RI), respectively. In vitro protein digestibility and starch hydrolysis kinetics were also evaluated to estimate the glycemic index, with conventional wheat bread serving as the reference. A consumer sensory evaluation involving 129 participants was also conducted to assess the acceptance of the developed bread. The results indicated that the pea-enriched bread exhibited substantially higher protein and dietary fiber content than the reference bread, reaching levels that allow the nutritional claims 'high protein content' and 'high fiber content'. Fatty acid analysis also revealed an improved in lipid profile, characterized by a higher proportion of polyunsaturated fatty acids, particularly omega-3 and omega-6 fatty acids. Fortification compensated for the naturally low lysine content of cereals, significantly increasing the Essential Amino Acid Index (EAAI). Regarding functionality, the enriched bread exhibited higher in vitro protein digestibility and a significantly lower estimated glycemic index than conventional bread, suggesting potential benefits for postprandial glycemic control. Although protein incorporation resulted in a firmer, darker crumb, sensory evaluation involving 129 consumers revealed good visual acceptance and a high purchase intention. These results highlight the potential of combining pea protein with natural fermentation as a viable strategy for developing nutritionally dense and healthier bakery products.

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MENU: Marine macroalgae: alternative recipes for a daily nutritional diet

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There is a growing interest in bioactive compounds produced by seaweeds that play a major therapeutic role in disease prevention in humans. Seaweed bioactive compounds include polysaccharides, pigments, fatty acids, minerals, polyphenols and peptides have been proven to possess various beneficial biological properties that could potentially contribute to health benefits[1]. These bioactivities are explored for functional properties such as antioxidant, antibacterial, anticancer, antidiabetic, antitumor, antiviral, anti-inflammatory and anticoagulant properties, both in in-vitro and in-vivo model system[2]. Marine natural products have the potential to be a rich source of innovative multitargeted medications for the prevention and treatment of many diseases, as well as associated complications. Several studies have shown various remedial effects of seaweeds' species against non-communicable diseases such as inflammation, obesity, diabetes, hypertension and viral infection. Here is stated novel food recipes developed under the project MENU that have in consideration novel food products with high nutritional value with the main ingredient seaweeds from the Portuguese coast, ingredients of no animal source and desserts without sugar, although sweet. All these conditions give a final product with advantages to the health of the consumer as present a high nutritious condition and bioactivities present in seaweeds. The recipes developed are present contributing to human health and wellbeing (Figure 1). Proprietary recipes are strictly confidential.



Figure 1 – Novel food recipes (desserts: mousse, puddings, sweet rice, jelly; velvety seaweed soups and rice with seaweed) developed at the project MENU.

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Targeting L-Cell Dopaminergic Signaling Through Precision Nutrition to Mitigate Age-Related Decline in Gut Endocrine Function

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The endocrine function of the gut is tightly regulated by nutritional cues, but this regulation becomes disrupted with ageing, leading to altered secretion of key metabolic hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY)[1]. These hormones are co-secreted by intestinal L-cells and play central roles in glucose homeostasis, appetite regulation, and energy metabolism. Dopamine, nearly half of which is produced in the gut, has emerged as an important modulator of GLP-1 signaling in peripheral tissues. However, the specific intestinal cell type responsible for dopamine production remains unidentified. The expression of dopamine receptors (DRs) and tyrosine hydroxylase (TH) - the rate-limiting enzyme in dopamine synthesis - in L-cells suggests that dopamine may act in an autocrine manner to regulate gut hormone secretion. Furthermore, adherence to the Mediterranean diet (MeD) has been associated with reduced mortality, lower risk of cardiometabolic diseases and cancer, and modulation of gut hormone secretion, including GLP-1 levels[2]. These observations underscore the important role of nutritional cues in regulating gut endocrine function.

This study investigated whether intestinal L-cells produce dopamine in response to dietary compounds, namely MeD bioactives and explored the role of dopamine in regulating GLP-1 and PYY secretion. Furthermore, the study explored how nutritional cues influence dopamine production in senescent L-cells and assessed the potential impact of ageing on L-cell dopaminergic signaling.

The human L-cell model NCI-H716 was exposed for 24 h to a panel of diet-derived bioactive compounds, including carvacrol, citral, silibinin, geraniol, sulforaphane, taxifolin, cinnamaldehyde, eucalyptol, and hydroxytyrosol (all at 5 µM). The dopamine precursor tyrosine (100 µM) and dopamine (0.5 µM) were also tested, alone or in combination with DRs inhibitors. The expression of TH, proglucagon, and PYY was analyzed, while GLP-1 secretion was quantified. To induce oxidative stress-mediated senescence, cells were treated with 200 µM H_2O_2 for 2 h, after which the medium was replaced and cells were cultured for a further 48 h. Senescence induction was assessed by measuring the expression of the senescence markers p53, p16, and p21. In addition, the expression of FOXA1, a transcription factor involved in L-cell maturation and hormone secretion, was evaluated. The expression of TH, proglucagon, and PYY, together with GLP-1 levels, was also assessed in senescent cells. Data are shown as mean ± SEM; significance was set at $p < 0.05$ (Student's t-test and ANOVA).

Treatment with all tested bioactive compounds increased TH expression after 24 h. In addition, exposure to dopamine and its precursor tyrosine significantly increased proglucagon and PYY expression. However, GLP-1 secretion into the conditioned medium remained unchanged. Dopamine-induced proglucagon expression was mediated through the D1 receptor, whereas the regulation of PYY expression involved the D4 receptor. Exposure to H_2O_2 for 2 h successfully induced cellular senescence, as evidenced by increased expression of the senescence markers p21 and p16. Senescence was associated with reduced expression of proglucagon, PYY, and FOXA1, while TH expression remained unchanged.

Overall, these findings suggest that dietary bioactive compounds modulate dopamine production in intestinal L-cells, and that dopamine contributes to the regulation of gut hormone expression. Targeting this pathway through precision nutrition may represent a novel strategy to mitigate age-related decline in gut endocrine function.

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Screening of important factors affecting Solid-state fermentation of apple and pear surpluses by Yarrowia species for protein enrichment

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The global transition towards more sustainable agri-food systems has intensified the need for innovative biotechnological approaches capable of valorizing agricultural surpluses and processing by-products. In this sense, reducing food loss and waste while reintegrating low-value biomass into the food chain has become a key priority from an environmental and economic perspective. In Portugal, pome fruit production represents an important agricultural sector, particularly through regionally certified products such as “Gala” and “Fuji” apples under Protected Geographical Indication (PGI) and “Rocha” pear under Protected Designation of Origin (DOP). Annual production exceeds approximately 150,000 tons; however, a fraction of this production is rejected before retail commercialization due to strict quality standards related to size, shape, or external appearance rather than nutritional composition[1]. Despite their preserved nutritional and biochemical properties, these rejected fruits remain underutilized, creating an opportunity for innovative valorization strategies yielding high-value food ingredients while promoting resource efficiency.

This work explores the use of solid-state fermentation (SSF) as an alternative strategy to upgrade apple and pear surpluses into protein-enriched ingredients with enhanced nutritional value. Given their naturally low protein content (<1.5% w/w), these substrates represent promising candidates for nutritional improvement through microbial bioprocessing[2]. The proposed approach employed selected yeast strains of the genus Yarrowia, including Y. lipolytica, a food-grade yeast with GRAS status and EFSA approval for specific food applications[3].

Preliminary experiments demonstrated that Y. lipolytica W29 successfully grew on the SSF of apple (Gala and Fuji) and Rocha pear. Bioprocessing significantly enhanced nutritional value, increasing five-fold the protein content and a 38% reduction in total sugars, yielding a product with an improved protein-to-sugar ratio.

A screening of several Yarrowia species and strains was carried out under submerged fermentation to assess their ability to grow on fructose-based medium and produce protein-rich biomass. Y. lipolytica W29 and Y. divulgata 5257/2 exhibited the most promising growth performance (DO600nm: 3.1 and 3.0, respectively), fructose consumption (0.2 g/L·h and 0.3 g/L·h, respectively), and total protein (31 % and 36 %, respectively). Additionally, Y. divulgata produced up to 5 g/L of mannitol, highlighting its biotechnological potential, as this compound is a valuable low-calorie sweetener widely used in the food and nutraceutical industries.

An optimization of SSF parameters (fermentation time, inoculum size, particle size of the substrate, moisture content, nitrogen supplementation, and yeast species) was performed using a Plackett–Burman experimental design to identify the most significant variables affecting fermentation performance. The results (Figure 1) indicated that moisture content and ammonium sulfate supplementation had the strongest positive effects on microbial biomass and protein production in SSF using apple and pear surpluses. In addition, among the yeast species evaluated, Yarrowia lipolytica W29 showed the best performance and was therefore selected for further experiments. Furthermore, inoculum size had a positive effect on protein production in apple (Gala) substrates, with higher inoculum concentrations leading to increased protein yields. Consequently, the highest inoculum concentration was selected for all substrates in subsequent experiments. Based on the overall Plackett–Burman results, a fermentation time of 4 days and a larger particle size (2.5–5 mm) were also selected for further experiments, as they were associated with improved fermentation performance. Finally, moisture content and ammonium sulfate concentration were selected for further optimization using a central composite design (CCD). The results demonstrate that SSF with Yarrowia spp is an effective route for enhancing the nutritional value of apples and pears, contributing to food waste reduction and the circular economy by enabling the valorization of fruit surpluses with high potential for new fruit-based food products.

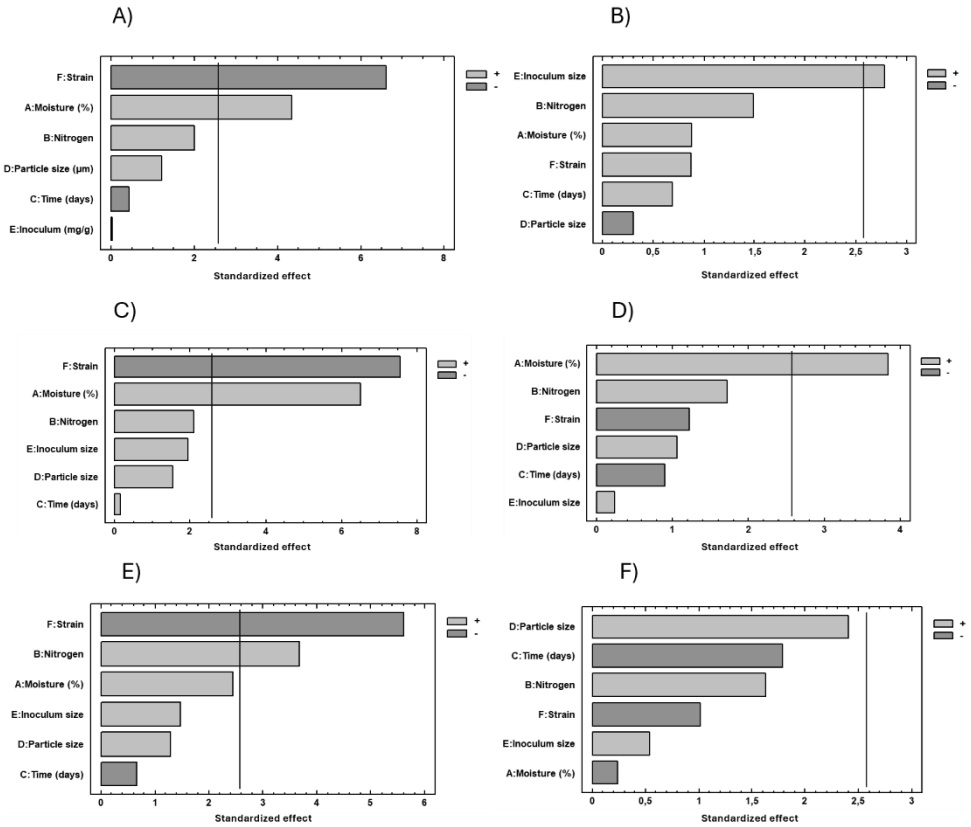


Figure 1 - Pareto chart illustrating the standardized effects of several solid-state fermentation variables (moisture content, nitrogen source, fermentation time, particle size, inoculum size, and Yarrowia spp. strains) on biomass production (left column) and total protein content (right column) of Gala apple (A, B), Fuji apple (C, D), and Rocha pear (E, F).

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Development of Sustainable Functional Meringues through the Upcycling of Fifth-Range Beetroot Surplus and Legume Aquafaba

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The transition towards sustainable food systems is promoting the valorization of agro-industrial side streams as natural alternatives to animal-derived ingredients and synthetic additives[1]. Beetroot powder (BP), produced by low-temperature drying of beetroot pomace obtained from surplus fifth-range vegetables, represents a valuable source of betalains and antioxidant compounds, whereas chickpea aquafaba (CA) and white-scarlet runner bean aquafaba (RBA), aqueous by-products of legume cooking, have emerged as promising plant-based foaming agents. However, to the best of our knowledge, their combined use in meringues has not been investigated. Therefore, this study investigated the effect of partially replacing icing sugar with BP (0, 5, 10 and 15%, w/w) in egg white (EW), CA and RBA meringues. The formulations were evaluated for their physicochemical, colour, textural and functional properties, and representative products are shown in Figure 1.

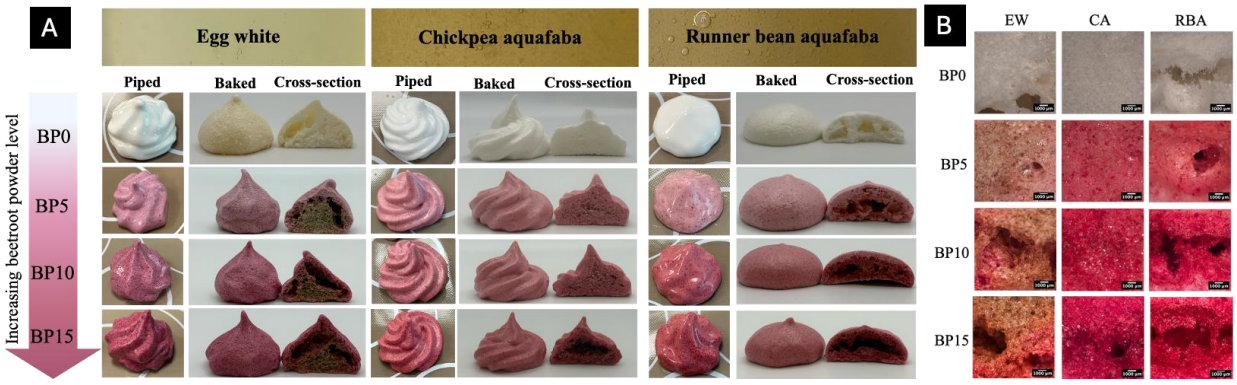


Figure 1. Representative appearance (A) and stereomicroscopic crumb structure (B) of EW, CA, and RBA meringues formulated with 0-15% BP (w/w sugar replacement).

BP concentration significantly affected the functional profile, producing a dose-dependent increase in bioactive compounds and antioxidant activity (BP effect, $p < 0.0001$). TPC increased from 81.1-90.4 to 258.2-294.4 mg GAE/100 g, total betalains reached 30.5 mg/100 g, and FRAP values increased from 2.73-6.29 to 16.67-20.70 µmol TE/g, depending on the foaming agent (FA). Instrumental colour analysis revealed a significant decrease in L* together with increasing a* and C* values ($p < 0.0001$), reflecting the progressive incorporation of beetroot pigments into the meringue matrix. Colour parameters were strongly associated with functional composition, with redness (a*) positively correlated with TPC ($r = 0.83$), total betalains ($r = 0.76$) and FRAP ($r = 0.85$), whereas lightness (L*) showed strong negative correlations with the same variables ($r = -0.85$ to -0.93). Compared with EW, aquafaba-based formulations (CA and RBA) exhibited improved colour retention, likely due to their lower pH (6.08 and 5.74 vs. 8.68 for EW) favored betalain stability while limiting non-enzymatic browning during baking. Representative and stereomicroscopic images (Figure 1) showed that increasing BP concentration progressively modified pore morphology and air-cell distribution. Consistently, fracture force significantly decreased (BP effect, $p < 0.0001$; FA × BP interaction, $p = 0.0124$), from 3.12 to 2.05 N in EW, from 2.36 to 1.51 N in CA, and from 2.31 to 1.57 N in RBA. The fiber-rich nature of BP likely interfered with foam development and air-cell stabilization at high substitution levels, leading to larger and more heterogeneous pores, particularly at 15% BP, which may compromise structural integrity and highlights the need to balance functional enrichment with technological performance. Overall, these findings highlight BP and aquafaba as valuable ingredients for the development of sustainable plant-based functional meringues, promoting the valorization of food processing side streams while naturally enhancing colour, fiber, mineral content, and antioxidant potential.

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Impact of Antibody Batch Variability on ELISA and Immunosensor Performance for Soybean Gly m TI Detection

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Soybean is one of the most important food allergens, <making its reliable detection in processed foods essential for consumer protection and regulatory compliance. Accurate detection of soybean allergens in processed foods remains a significant challenge due to the effects of food processing and matrix complexity on analytical performance[1]. The reliability of immunoassays is further dependent on the consistency and quality of the antibodies used, yet the impact of antibody batch variability is rarely investigated. In this work, the influence of antibody batch variability on the detection of soybean Gly m TI allergen, was investigated using two different biorecognition platforms: indirect enzyme-linked immunosorbent assay (ELISA) and an electrochemical immunosensor.

Initially, an indirect ELISA was developed, using a specific anti-Gly m TI IgG, and optimized by assessing protein extraction conditions, antibody concentration, and analytical working range. The optimized method was applied to model food systems representative of biscuits, sausages, and cooked hams containing soybean-derived ingredients subjected to different processing conditions and compared to previously developed immunosensor for Gly m TI detection. Gly m TI was successfully detected in all tested matrices, demonstrating the applicability of the assay to a variety of food products. Nevertheless, the method exhibited a relatively high limit of detection (LOD) of 1,000 mg/kg, irrespective of matrix composition or processing treatment, which prompted a detailed investigation of commercial antibody batch variability. In this context, four independent batches of polyclonal anti-Gly m TI antibodies (Ab1 – Ab4), produced either in the same year or five years apart, were evaluated for their analytical performance by ELISA and an electrochemical immunosensor[2]. Antibodies generated during the same production period exhibited comparable behavior, showing similar sensitivity, working range, and optimal dilution factors. However, differences were observed between antibodies produced five years apart. The batches obtained in 2019 (Ab1 and Ab3) consistently demonstrated superior analytical performance compared with those produced in 2024 (Ab2 and Ab4)[3].

Using Ab1 and Ab3, the developed assays achieved broader linear ranges and enabled the detection of Gly m TI at concentrations as low as 0.1 mg/kg in raw and processed food matrices, including biscuits, cooked ham, and autoclaved sausages. These antibody batches also required substantially lower working concentrations to generate equivalent analytical responses. In contrast, the reduced affinity exhibited by Ab2 and Ab4 negatively affected assay sensitivity and overall performance. Similar trends were observed when these antibodies were incorporated into electrochemical immunosensors, confirming that antibody quality was a critical determinant of analytical efficiency regardless of the detection platform employed[2,3].

Overall, the results demonstrated the strong performance of indirect ELISA for Gly m TI determination, while emphasizing the importance of monitoring antibody batch variability during assay development and validation. The study further highlights the potential of electrochemical immunosensors as complementary tools for allergen analysis and represents, to the best of our knowledge, the first systematic comparison of multiple antibody batches across both ELISA and biosensor-based detection strategies. These findings provide valuable insights for the future design of robust immunoanalytical methods, including multiplexed allergen detection and high-throughput screening applications.

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Workflow de Análise Sensorial e Modelos Celulares Orais para Compreensão do Sabor em Alimentos de Origem Vegetal

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A compreensão da forma como os consumidores percecionam o sabor constitui um pilar fundamental do design alimentar inovador e sustentável, particularmente no desenvolvimento de alimentos de origem vegetal, nos quais o amargor e a adstringência representam barreiras comuns à aceitação. Este trabalho apresenta uma abordagem integradora e inovadora, que abre novos caminhos para um design alimentar centrado no consumidor, ao estabelecer uma ligação entre a perceção sensorial humana e a caracterização de parâmetros fisiológicos/biológicos humanos e de interações à escala molecular.

A metodologia desenvolvida combina ensaios bioquímicos in vitro avançados, modelos celulares orais e estudos de interações moleculares com dados sensoriais in vivo e a caracterização do perfil salivar humano, centrando-se no modo como as diferenças interindividuais nas proteínas salivares e na fisiologia oral influenciam a perceção de alimentos ricos em polifenóis. Ao reunir disciplinas como a ciência sensorial, a fisiologia oral, a biologia molecular e a química alimentar, este quadro conceptual permite um mapeamento sem precedentes da forma como compostos alimentares específicos – nomeadamente os polifenóis – interagem com constituintes orais, como as proteínas salivares e as superfícies mucosas. De forma crucial, este estudo explora ainda como estas interações se traduzem em respostas percetivas, como a adstringência e o amargor.

Este estudo sublinha a necessidade de integrar dados moleculares e sensoriais para a inovação alimentar orientada para o consumidor, abrindo caminho à personalização de perfis de sabor em dietas promotoras de saúde, como a dieta mediterrânica ou as alternativas de base vegetal. Este trabalho tem implicações relevantes para a melhoria da aceitação e da adesão a padrões alimentares sustentáveis.

Palavras-chave: saliva, modelos cavidade oral, adstringência

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Nutritional profile and lutein content of functional bread enriched with honey *Chlorella vulgaris*

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In recent decades, the increasing incidence of chronic non-communicable diseases has been associated with unhealthy lifestyles and the excessive consumption of ultra-processed foods. In this context, the development of food products that promote a balanced diet and are enriched with functional ingredients has become a major trend in the food industry, with bread emerging as one of the most promising delivery vehicles due to its widespread consumption and versatility[1,2]. Among promising bio-based ingredients, the microalga *Chlorella vulgaris* stands out for its protein, fiber, vitamin, and mineral contents, as well as for being a rich source of lutein, a carotenoid associated with several benefits for macular health [3]. Thus, the present study aimed to develop an innovative bread enriched with 5 and 10% *C. vulgaris* and to evaluate the effects of its incorporation on the nutritional composition and lutein content of the final product. Preliminary sensory evaluation guided the selection of the most acceptable formulation, which was assessed through a consumer acceptance test using a nine-point hedonic scale involving 123 untrained participants. The nutritional characterization of the bread was performed according to official AOAC methods. The lutein content was determined by high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD) after exhaustive ultrasound-assisted extraction with ethanol. All analyses were performed in triplicate, and results are expressed as mean ± standard deviation. Statistical differences between formulations were evaluated using an independent-samples Student's t-test ($p < 0.05$). A bread without *C. Vulgaris* addition was also developed as the control. Preliminary sensory evaluation indicated that bread enriched with 5% *C. vulgaris* did not significantly affect the product's flavor, whereas the 10% formulation exhibited a more intense and perceptible flavor. Bread fortified with 5% *C. vulgaris* presented a moisture content of 24 ± 1 %, an ash content of 2.2 ± 0.1 g/100 g fresh weight (fw), a lipid content of 0.277 ± 0.007 g/100 g fw, a protein content of 9.0 ± 0.3 g/100 g fw, a dietary fiber content of 4.5 ± 0.2 g/100 g fw, and a carbohydrate content of 60 ± 1 g/100 g fw. In comparison, the control bread presented a moisture content of 27.8 ± 0.7%, an ash content of 1.6 ± 0.2 g/100 g fw, a lipid content of 0.20 ± 0.01 g/100 g fw, a protein content of 8.0 ± 0.3 g/100 g fw, a dietary fiber content of 3.6 ± 0.6 g/100 g fw, and a carbohydrate content of 58.7± 0.6 g/100 g fw. Compared with the control, bread enriched with 5% *C. vulgaris* showed significantly higher ash, lipid, protein, and energy contents ($p < 0.05$), whereas no significant differences were observed for dietary fiber or carbohydrate content. Fatty acid analysis revealed that bread enriched with 5% *C. vulgaris* exhibited a predominantly unsaturated fatty acid profile, including the omega-3 fatty acids eicosapentaenoic acid (EPA), which was not detected in the control bread. Lutein quantification revealed a content of 1.25 µg/g dry weight (dw) in bread enriched with 5% *C. vulgaris*, whereas this carotenoid was not detected in the control bread. These findings highlight the potential of *C. vulgaris* as an innovative ingredient for the development of sustainable and nutritious bakery products.

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Modulação do Equilíbrio Ácido–Base das Antocianinas por Proteínas: Uma Nova Estratégia para a Formação de Cor Azul Estável

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A obtenção de corantes azuis naturais estáveis constitui um dos principais desafios da indústria alimentar. Embora as antocianinas sejam candidatos promissores, a formação de tonalidades azuis requer normalmente estruturas altamente aciladas ou complexação com metais. Neste trabalho foi desenvolvida uma estratégia alternativa baseada na interação não covalente entre proteínas e antocianinas, permitindo obter uma coloração azul intensa e estável.[1]

Como sistema modelo utilizaram-se a malvidina-3-O-glucósido (M3G) e a albumina sérica bovina (BSA). A interação foi estudada entre pH 6 e 9 por espectroscopia UV-Vis, calorimetria de titulação isotérmica, ensaios de competição, modelação molecular e simulações de dinâmica molecular. A estabilidade da cor e da antocianina foi posteriormente avaliada durante o armazenamento.

A presença de BSA promoveu deslocamentos batocrómicos e hipercrómicos significativos, originando uma intensa coloração azul em meio alcalino. Os resultados demonstraram que a proteína interage preferencialmente com a forma quinoidal dianiônica da antocianina, deslocando o equilíbrio ácido-base para esta espécie, responsável pelas tonalidades azuis (**Figura 1**). A interação revelou-se predominantemente governada por forças eletrostáticas e ligações de hidrogénio. A pH 9, o complexo proteína–antocianina apresentou elevada estabilidade, sem perdas significativas de cor ou de antocianina após 28 dias de armazenamento a 5 °C.

Este trabalho demonstra que a complexação proteína–antocianina constitui uma estratégia inovadora para promover e estabilizar a cor azul em antocianinas, abrindo novas perspetivas para o desenvolvimento de corantes naturais destinados à indústria alimentar.

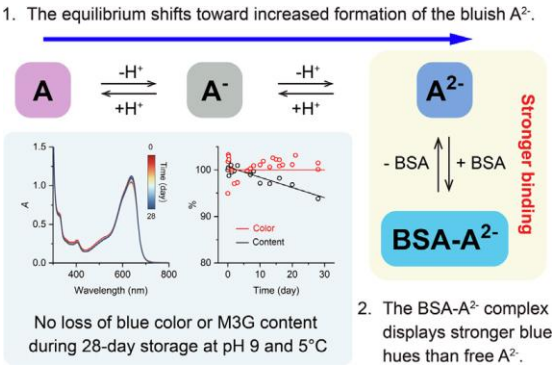


Figure 1 - A complexação proteína–antocianina desloca o equilíbrio ácido–base para a forma quinoidal dianiônica, promovendo uma coloração azul intensa e estável.

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Porcine cartilage by-product as valuable technological ingredient in food

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Porcine cartilage is a by-product of meat industry, with potential high value due to its unexploited content of glycosaminoglycans and protein, mostly collagen. Glycosaminoglycans are negatively charged polysaccharides constituted by a disaccharide repetitive unit of galactosamine or glucosamine and L-iduronic acid or D-glucuronic acid. These polysaccharides are commonly attached to core proteins, forming proteoglycans. The search for these compounds is increasing due to the possible effects on health and more. So, the aim of this study is to use a defatted extract of porcine cartilage that can be used in food applications, valorizing this by-product.

Porcine cartilage contains many microorganisms, so the cartilage was cooked first at 65ºC for 30 minutes and after to inactivate endogenous enzymes at 95ºC for 15 minutes. This step helps the griding and drying process of porcine cartilage with a weight loss of 57,4%. This powder of porcine cartilage containing fat, that if oxidated releases unpleasant smell that could comprise its integration in food systems, needs to be defatted. So, the extract was stirred with hexane and filtrated to remove the fats, with weight loss of 47%. To characterize the defatted extract, the neutral sugars and hexosamine, as alditol acetates, were analyzed by GC-FID and GC-MS, respectively, and uronic acids by *m*-phenylphenol colorimetric method. These methods confirmed the presence of glycosaminoglycans (5%) in the extract of porcine cartilage defatted. Also, the protein content was analyzed by elemental analysis and the amino acid composition with GC-MS, glycine, hydroxyproline and glutamate/glutamic acid the major components. The extract contains glycosaminoglycans and protein that could have the right properties to be used in ham matrix, so we study the replacement of carrageenan in the cooked ham formulation with the defatted extract. Texture Profile Analysis of the cooked ham model revealed no differences between the ham with carrageenan and with porcine cartilage defatted extract, that could indicate that this extract containing protein and glycosaminoglycans could be used to development of new food technological ingredients.

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Meat alternative proteic foods viability study with red algae carrageenan ingredients

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Carrageenan refers to a group of sulphated galactans derived from red algae. These polysaccharides are commonly used to enhance the textural properties of meat products and their analogues. Meat substitutes typically depend on protein gelation or other hydrocolloids to provide the firmness and textural characteristics required to mimic conventional meat products. Although vegetarian formulations can include animal-derived ingredients, such as egg whites due to their gelling properties, vegan alternatives depend on plant-based proteins supported by carbohydrate-based hydrocolloids capable of forming stable and robust gel networks.

This study examined the characteristics of carrageenans suitable for incorporation into two distinct protein-based food formulations: cooked ham and a meat-free analogue. In the cooked ham formulation, a commercially available carrageenan was used to improve water-holding capacity by forming a gel that binds water, reduces shrinkage, and increases yield. Its carbohydrate composition consisted of 36 mol% galactose and 19 mol% 3,6-anhydrogalactose, with 41mol% sulphate esters, accounting for a total carbohydrate content of 34%. Texture Profile Analysis of the cooked ham model revealed that carrageenan addition significantly increased hardness from 83.1 N to 97.0 N and chewiness from 2474 N to 3239 N compared to the carrageenan-free formulation. This increase in hardness and chewiness is consistent with gel formation, resulting in a more sliceable product. For the cooked ham analogue, gel emulsion formation was achieved using a commercial carrageenan-rich ingredient composed of 65% carbohydrates (39 mol% galactose, 18 mol% 3,6-anhydrogalactose, and 20 mol% glucose), with 22 mol% of sulphate esters. The final formulation exhibited a firm product with desirable textural properties, similar to a vegetarian version formulated with egg white as a gelling agent.

The results of this study reveal that carrageenan represents a promising gelling hydrocolloid for use in cooked ham and their analogues formulations.

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Green Tea Extract as Functional Food Ingredients for the Selective Modulation of Probiotic and Pathogenic Adhesion

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The adhesion of microorganisms to the intestinal mucus layer is a critical determinant of successful colonisation and persistence within the gastrointestinal tract. This process is particularly relevant for probiotic bacteria, whose ability to adhere to mucosal surfaces can enhance their competitive fitness, promote host-microbe interactions, and contribute to gut health. Conversely, the adhesion of pathogenic bacteria represents a key step in colonisation and infection, making the selective modulation of microbial adhesion an attractive strategy for supporting intestinal homeostasis.

Plant-derived polyphenols have emerged as promising bioactive compounds capable of influencing microbial behaviour within the gut ecosystem. Among these, green tea catechins have been extensively studied for their antimicrobial, antioxidant, and anti-inflammatory properties. Increasing evidence suggests that these compounds may exert selective effects on microorganisms, inhibiting pathogenic species while preserving or even promoting beneficial bacteria. However, their influence on microbial adhesion under competitive conditions remains poorly understood.

This study evaluated the impact of a commercial catechin-rich green tea extract (Sunphenon® 90LB) on the viability and adhesion of probiotic and potentially pathogenic bacteria to mucin/bovine serum albumin (BSA)-coated surfaces designed to mimic the intestinal environment. The probiotic strains included Lacticaseibacillus casei 01, Lactobacillus acidophilus LaLA-5, Bifidobacterium animalis Bo, and B. animalis subsp. lactis BB-12, while Escherichia coli NCTC 9001 was used as a model intestinal pathogen. Minimum inhibitory and bactericidal concentrations were determined, followed by viability and adhesion assays conducted in both monoculture and probiotic-pathogen co-culture systems.

The green tea extract exhibited selective antimicrobial activity. E. coli showed the highest susceptibility, with a minimum inhibitory concentration of 8 mg/mL and a minimum bactericidal concentration of 10 mg/mL. In contrast, both Bifidobacterium strains were unaffected at the concentrations tested, while L. casei and L. acidophilus displayed only limited sensitivity. Viability assays confirmed a marked reduction in E. coli populations following exposure to the extract, whereas probiotic strains largely maintained their viability. Adhesion studies revealed that the extract significantly reduced the attachment of E. coli to mucin/BSA-coated surfaces. In monoculture systems, probiotic adhesion was only marginally affected, with Bifidobacterium strains maintaining adhesion levels comparable to untreated controls. Similar trends were observed in co-culture experiments, where the extract consistently reduced pathogen adhesion while preserving, and in some cases enhancing, probiotic adhesion. Notably, B. animalis Bo exhibited improved adhesion in the presence of both the extract and E. coli, suggesting a potential synergistic interaction between green tea polyphenols and specific probiotic strains.

Overall, the results demonstrate that catechin-rich green tea extracts can selectively inhibit pathogen viability and adhesion while preserving beneficial microbial populations. These findings support the potential application of green tea polyphenols as functional food ingredients capable of promoting a healthier gut microbiota through the selective modulation of microbial colonisation processes.

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Fibre-rich fraction as the structural reservoir of antioxidant phytochemicals in sweet potato flour

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Sweet potato flour, particularly from orange- and purple-fleshed cultivars rich in carotenoids and anthocyanins, is gaining relevance as a gluten-free functional ingredient and natural colourant source1. However, the physical distribution of these antioxidant phytochemicals within the flour matrix and the structural components governing their retention remain insufficiently understood, limiting the rational optimisation of processing for enhanced stability and bioaccessibility.

This study aimed to determine the structural localisation of carotenoids and anthocyanins in hot-air-dried sweet potato flour by fractionating the flour into starch-rich and fibre-rich fractions and characterising phytochemical-matrix associations. Two pigmented cultivars, Bellevue (orange-fleshed, carotenoid-rich) and NP1648 (purple-fleshed, anthocyanin-rich), were selected as phytochemical-rich matrices, while a white-fleshed cultivar (Bonita), largely devoid of these pigments, was included as a non-pigmented structural reference. Flours were produced by hot-air drying (75 °C, 5 h) and subsequently fractionated by wet extraction. The resulting slurry was filtered through cotton cloth; the retained insoluble residue was collected as the fibre-rich fraction, while the filtrate was allowed to settle to recover the starch-rich fraction. Both fractions were dried at 40 °C, milled and sieved to obtain a uniform particle size. Total carotenoid content (TCC; mg β-carotene equivalents/100 g db) was measured spectrophotometrically at 470 nm following acetone: ethanol extraction, and total anthocyanin content (TAC; mg cyanidin-3-glucoside equivalents/100 g db) was quantified using the pH differential method. TCC and TAC were determined in whole flours and both fractions of the two pigmented cultivars. Phytochemical-matrix interactions and structural morphology were further investigated by scanning electron microscopy (SEM) and attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR; 4000-400 cm-1). Principal component analysis (PCA) was applied to the fingerprint region (1801-899 cm-1) across all three cultivars.

Antioxidant phytochemicals were preferentially and consistently retained in the fibre-rich fraction, which accounted for 57–71% of the total carotenoid and anthocyanin content. ATR-FTIR supported this observation by revealing distinct compositional differences between fractions. The band near 1520 cm-1, associated with aromatic skeletal vibrations of phenolic compounds, was more intense in NP1648, particularly in its fibre-rich fraction. Furthermore, the ester carbonyl band at ≈1740 cm-1, consistent with esterified cell-wall components, was detected exclusively in the fibre-rich fractions of both pigmented cultivars. PCA of the FTIR spectra (explaining 95% of total variance) positioned whole-flour samples closer to the fibre-rich fraction, indicating that this fraction largely governs the overall spectroscopic fingerprint of the flour. SEM analysis further revealed a clear structural dichotomy: the starch-rich fraction consisted predominantly of smooth, rounded starch granules with limited interaction surface area; in contrast, the fibre-rich fraction exhibited irregular, rough, and heterogeneous structures containing abundant cell-wall remnants, providing a substantially greater surface area and a structurally complex matrix capable of retaining phytochemicals.

These findings support a mechanistic basis for the preferential retention of phytochemicals within the fibre-rich fraction. Cell-wall polysaccharides, including cellulose, hemicelluloses and pectins, may provide numerous hydroxyl and carboxyl functional groups capable of establishing hydrogen bonding, hydrophobic interactions and other non-covalent associations with antioxidant phytochemicals 2,3. In addition, hydroxycinnamic acids are known to occur esterified to cell-wall polysaccharides, while pigments may also remain physically entrapped within the cell-wall architecture.

Overall, this study demonstrates that pigmented sweet potato flour is a structurally compartmentalised system rather than a homogeneous matrix, in which the fibre-rich fraction acts as the primary structural reservoir of antioxidant phytochemicals. These findings provide a mechanistic basis for understanding phytochemical retention within the sweet potato matrix and offer valuable insights for designing processing and fractionation strategies aimed to enhancing

phytochemical stability, modulating digestive release and improving the functional performance of pigmented sweet potato flours in food application.

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Beyond extraction: Optimizing Protein Recovery from Crustaceans and Tree Nuts Towards Assessing their Allergenic Potential

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The efficient extraction and purification of food proteins are essential for their subsequent characterization and application in food chemistry and allergen research. However, protein recovery and integrity can be significantly affected by extraction conditions and buffer composition, particularly in complex biological matrices. This study aimed to optimize protein extraction and purification protocols for a diverse selection of crustaceans and tree nut species, by evaluating the influence of extraction conditions and buffer systems on protein recovery.

Protein extracts were obtained from eight crustacean species (Cancer Pagurus, Pleoticus Muelleri, Litopenaeus Vannomei, Homorus Gammarus, Mytilus Edulis, and Solen Marginatus) and six tree nut species (Brazil nut, pecan nut, macadamia nut, walnut, almond, and cashew). Two extraction approaches were evaluated: incubation at 60 °C for 2 h and overnight extraction at 4 °C. Seven different extraction buffers (PBS 0.2 M pH 7.5, Tris-HCl 0.1 M pH 8.0, Tris-HCl 0.1M + 4% SDS pH 7.6, NH4HCO3 0.1 M pH 8.5, H2O pH 9.0, H2O pH 12.0, and ascorbic acid 9.5 mM pH 2.5) were tested for each sample. Protein concentration was determined spectrophotometrically, and extraction performance was assessed based on protein yield by SDS-PAGE. The most suitable extraction conditions for each species were selected and subsequently subjected to purification using Amicon® ultrafiltration filters with different molecular weight (MW) cut-offs (10, 30, 50 and 100 kDa) or with dialysis membranes (7 MWCO). The performance of purification strategies was evaluated by SDS-PAGE (5-12%) and immunoblotting in reducing and non-reducing conditions using sera from tree nut or crustacean allergic patients.

Protein recovery was strongly influenced by both extraction conditions and buffer composition, highlighting the importance of matrix-specific optimization. Among the tested conditions, overnight extraction at 4 °C using a Tris-HCl-SDS buffer provided the highest protein recovery for most of the crustacean and tree nut species analyzed. The combination of mild extraction conditions and detergent-assisted solubilization enhanced protein extractability while minimizing protein structural modifications. Although species-dependent differences were observed, Tris-HCl-SDS consistently outperformed the remaining extraction buffers across most matrices. In contrast, ultrafiltration using Amicon® devices was effective for sample concentration but showed limited capability for protein fractionation. SDS-PAGE and immunoblotting results revealed substantial overlap between retained and filtrate fractions, indicating insufficient separation of proteins based on molecular weight alone. Dialysis allowed purifying proteins around 37-40 kDa, which are probably tropomyosins in the case of crustacean species.

This study establishes optimized extraction conditions for proteins from a diverse range of crustaceans and tree nut species and demonstrates the strong influence of extraction parameters on protein recovery. While ultrafiltration provided sample concentration, it was not effective as a stand-alone strategy for protein separation. The findings support the need for complementary purification approaches, such as chromatographic techniques, to achieve efficient protein fractionation in complex food matrices.

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Characterization of the nutritional composition of four macroalgae species from the Western Portuguese coast to food diet alternatives

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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 protein, fatty acids and polysaccharide and uronic acids 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.



Figure 1 – Species studied in this work (from the left to the right): *Calliblepharis jubata*, *Osmundea pinnatifida*, *Undaria pinnatifida* and *Fucus vesiculosus*.

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Nutritional Enrichment of Domestic Crickets (*Acheta domesticus*) Using DHA-Rich Microalgae and Agro-Industrial By-Products

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The increasing demand for sustainable and nutritionally rich food sources has intensified interest in edible insects as alternatives to conventional livestock production. Among edible insects, the house cricket, Acheta domesticus, is recognized for its high protein content, efficient feed conversion, and low environmental impact. Typically reared on by-products from vegetable processing industries, crickets represent a promising resource within circular and sustainable food systems. However, despite their excellent protein quality, their fatty acid composition—particularly omega-3 content—can be further improved to meet the nutritional requirements of specific consumer groups, including athletes and the elderly[1].

This exploratory project aimed to enhance the lipid fraction of Acheta domesticus by enriching it with omega-3 highly unsaturated fatty acids (HUFA), namely eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA), through dietary manipulation. DHA is especially relevant due to its recognized role in brain development, cardiovascular health, antiinflammatory activity, and prevention of neurodegenerative diseases.

To achieve this objective, two experimental diets were formulated by incorporating 5% and 10% DHA-rich microalgae into a standard cricket feed, partially replacing the baseline diet composed of agro-industrial by-products. Crickets were reared under controlled environmental conditions, and their biochemical composition was evaluated at the subadult stage. The study focused particularly on lipid content and fatty acid profile characterization[2].

The results demonstrated that dietary supplementation with DHA-rich microalgae significantly improved the lipid profile of the crickets (Figure 1). Although saturated fatty acids (SFA) remained the predominant group, high levels of polyunsaturated fatty acids (PUFA) were also observed. The fatty acid distribution was consistent with previous studies, showing the prevalence of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), and linoleic acid (18:2 n-6). Polyunsaturated fatty acids were mainly represented by n-6 fatty acids, especially 18:2 n-6. Importantly, EPA and DHA levels, absent or negligible in the control group, increased following the incorporation of microalgae into the diets. An increase in SFA content and a reduction in monounsaturated fatty acids (MUFA) were also observed.

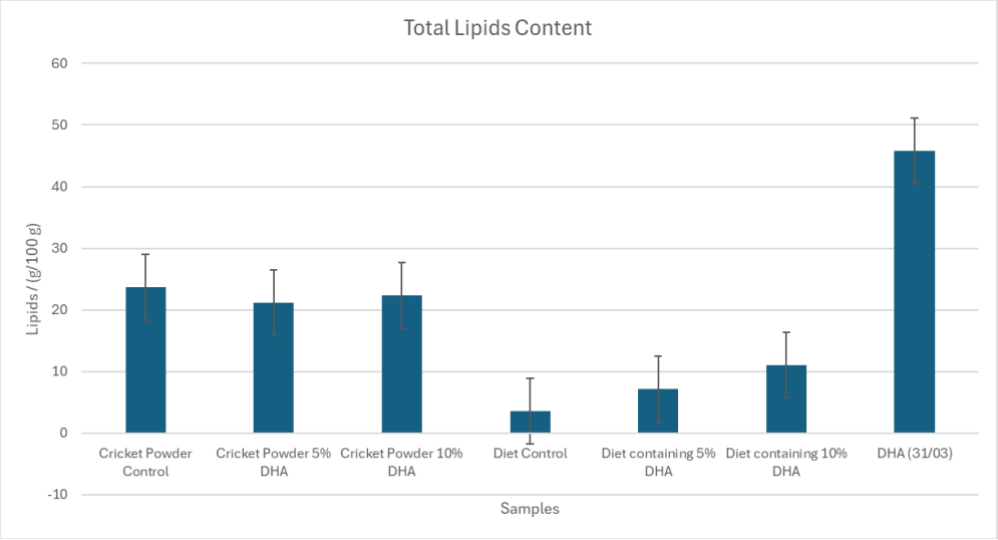


Figure 1 – Total lipid content.

Beyond improving cricket nutritional quality, this work contributes to the development of innovative insect-based functional foods. The enriched lipid extract obtained from the crickets is intended for incorporation into novel spreadable water-in-oil cream formulations reinforced with healthy lipids. These products, combining cricket protein and lipid isolates, represent an innovative approach within sustainable food technology and functional food development.

By integrating insect farming with algal biotechnology, this strategy provides a scalable and cost-effective solution for enhancing emerging protein sources without compromising sustainability. Future work will investigate the effects of diets supplemented with seaweed from the Portuguese coastline on both lipid and protein composition, as well as the amino acid profile of cricket powder.

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Microwave-assisted extraction of phenolic compounds and flavonoids from maritime pine (*Pinus pinaster*) bark: effect of solvent and process conditions, with a view to application in traditional sausages

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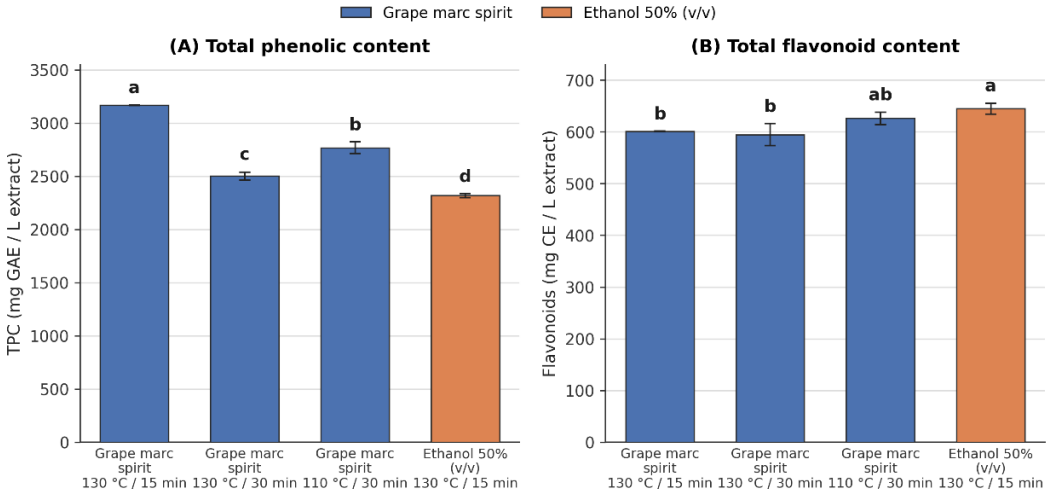
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Pine bark (Pinus pinaster) is an abundant forestry by-product in Portugal recognised for its richness in bioactive compounds, particularly phenolic compounds and flavonoids, with documented high antioxidant capacity [1]. These extracts are enriched in proanthocyanidins and condensed tannins, compounds to which much of the reported antioxidant activity is attributed. The growing interest in replacing synthetic antioxidants with natural alternatives, combined with valorisation of agro-forestry residues in a circular economy framework, drives research into new sources for food applications [2]. Traditional sausages, owing to their high fat content, are susceptible to lipid oxidation during processing and storage, compromising product quality and shelf life. The present work aimed to optimise production and characterisation of pine bark extracts rich in phenolic compounds, obtained by microwave-assisted extraction (MAE), as a preliminary step towards their application in traditional sausages as natural food additives. MAE has emerged as a green extraction technique that is faster, more efficient and uses less solvent than conventional methods, making it attractive for recovery of bioactive compounds from plant matrices [3].

MAE was applied to pine bark under four distinct conditions, varying solvent, temperature and time: grape marc spirit at 130 °C for 15 min, grape marc spirit at 130 °C for 30 min, grape marc spirit at 110 °C for 30 min, and ethanol 50% (v/v) at 130 °C for 15 min. The experimental design evaluated the effect of solvent and process parameters on target compound recovery. Total phenolic content (TPC) was quantified by the Folin–Ciocalteu method (mg gallic acid equivalents per litre, mg GAE/L), and flavonoid content by aluminium chloride colorimetric method (mg catechin equivalents per litre, mg CE/L). All determinations were performed in triplicate. After verifying normality (Shapiro–Wilk test) and variance homogeneity (Levene's test), results were compared using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Figure 1 presents total phenolic and flavonoid contents of Pinus pinaster bark extracts, illustrating differential responses to extraction conditions. Total phenolic content ranged from 2318.52 ± 18.52 mg GAE/L (ethanol 50%, 130 °C, 15 min) to 3167.59 ± 0.93 mg GAE/L (grape marc spirit, 130 °C, 15 min), with all four conditions differing significantly ($p < 0.001$). Extraction with grape marc spirit at 130 °C/15 min maximised TPC, whilst extending time to 30 min decreased phenolic content.



Bars: mean ± SD (n = 3). Within each panel, bars labelled with different letters are significantly different (one-way ANOVA + Tukey HSD, $p < 0.05$).

Figure 1 – Total phenolic (A) and flavonoid (B) contents of *Pinus pinaster* bark extracts obtained by MAE. Mean ± SD (n = 3); different letters denote significant differences (ANOVA + Tukey HSD, $p < 0.05$).

Flavonoid content ranged from 594.67 ± 21.25 to 644.75 ± 10.25 mg CE/L. Ethanol 50% showed the highest flavonoid value, significantly greater than grape marc spirit at 130 °C ($p = 0.006$). Grape marc spirit at 110 °C/30 min showed an intermediate position with no significant differences from other conditions. These results demonstrate that both compound classes respond distinctly to solvent and extraction conditions, reflecting process selectivity: grape marc spirit favoured total phenolic recovery, whilst aqueous ethanol proved more effective for flavonoid extraction. In conclusion, pine bark is a highly promising renewable source of bioactive compounds, with composition strongly dependent on MAE conditions. Extraction with grape marc spirit at 130 °C/15 min produced extracts rich in total phenolics, whilst ethanol 50% was most effective for flavonoid-enriched extracts. Grape marc spirit, a food-grade solvent, does not introduce toxic residues and aligns with clean-label approaches. The extracts demonstrate potential for application in traditional sausages and cured meat products as natural additives, contributing to improved oxidative stability and shelf life whilst advancing valorization of this forestry by-product. Future work should evaluate these conditions in real food systems, considering complementary parameters such as total antioxidant activity and extraction yield optimisation.

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[P.3.7]

Pre-fermentation addition of *Pinus pinaster* Aiton subsp. *atlantica bark* extract enhances the phenolic content and antioxidant capacity of beer during storage

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The brewing sector is innovating rapidly, with growing demand for differentiated beverages that combine sensory appeal with functional value; antioxidant-rich non-cereal adjuncts have emerged as a promising route to such functional beers[1]. Maritime pine (Pinus pinaster Aiton subsp. atlantica) bark, an abundant forestry by-product in northern Portugal, constitutes a rich source of polyphenols, predominantly proanthocyanidins, phenolic acids and flavonoids, which possess well-documented antioxidant properties[2]. Despite this potential, its application in brewing remains scarcely explored, the challenge being to enrich the beverage without compromising fermentation or stability. This study evaluated the incorporation of a pine bark extract (PBE) as a functional ingredient in beer, assessing its effect on phenolic content and antioxidant capacity and their retention during storage.

PBE, obtained by microwave-assisted extraction with a 48% (v/v) hydroethanolic solution (grape marc spirit), was incorporated at 37 mL/L (below the toxicologically established safety threshold) under three conditions: control (no PBE), pre-fermentation and post-fermentation addition. Beers were analysed for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH, ABTS, ORAC, FRAP) over 60 days (0, 30, 60). Differences were assessed by one-way ANOVA with Tukey's HSD test ($p < 0.05$), after confirming normality (Shapiro-Wilk) and homogeneity of variance (Levene), and a PCA biplot was performed to explore the overall variation among samples.

Both PBE beers significantly outperformed the control. At day 0, TPC rose from 239 mg GAE/L in the control to 262 and 257 mg GAE/L in the pre- and post-fermentation beers, and TFC from 12.1 to 17.1 and 16.3 mg catechin/L, respectively ($p < 0.001$); antioxidant capacity followed the same trend across all assays, with ORAC reaching 11.9 µmol TE/mL in the pre-fermentation beer against 6.6 in the control (DPPH, ABTS and FRAP also higher, $p < 0.05$). This enrichment was largely retained during storage: the control showed the steepest loss of TPC ($p < 0.001$), whereas the pre-fermentation beer remained stable. The assays nonetheless diverged over time, ORAC declining sharply by day 30 while ABTS increased, likely reflecting assay-specific chemistry and the transformation of phenolics during maturation. These patterns were confirmed by PCA (Figure 1), whose first two components explained 83.1% of the variance (PC1, 52.2%; PC2, 30.9%). PC1 separated the control (negative side) from both PBE beers (positive side, towards the TPC, TFC and DPPH vectors), while PC2 reflected storage time, with day-0 samples aligned with ORAC migrating towards ABTS and FRAP by day 60. The 95% confidence ellipses of the control and PBE beers barely overlapped along PC1, giving multivariate support to the univariate differences and identifying pre-fermentation addition as the richest and most distinct profile, consistent with earlier reports on pine-supplemented brewing[3].

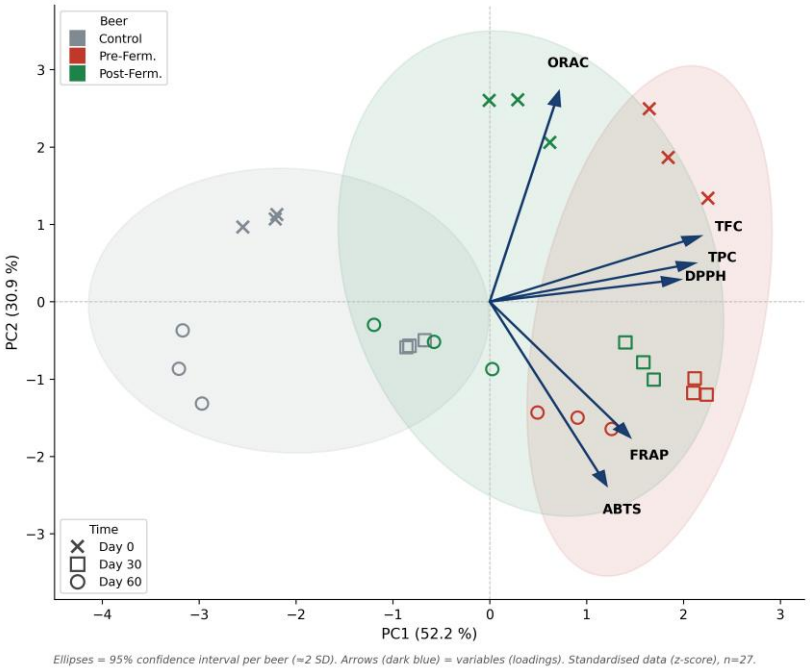


Figure 1 – PCA biplot of the beers' phenolic and antioxidant profile.

Overall, the results demonstrate that Pinus pinaster bark extract can substantially enhance the phenolic content and antioxidant capacity of beer at a safe incorporation level, and that this benefit persists for at least 60 days. The superiority and greater stability of the pre-fermentation treatment identify it as the preferred strategy for a functional beer with a sustained antioxidant reserve, while the contrasting assay trajectories underline the value of a multi-assay approach. Beyond the technological gain, the use of a regional forestry by-product aligns with circular-economy principles and the valorisation of endogenous resources.

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Lupin, Grass Pea and Chickpea Flours as Milk-Protein Replacers in Hybrid Liver Pâté: Technological and Sensory Effects

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Hybrid meat products can support the diversification of protein sources while preserving familiar product formats[1]. This study evaluated the weight-for-weight replacement of the milk-protein ingredient in liver pâté with white lupin (Lupinus albus; LA), grass pea (Lathyrus sativus; LS) or chickpea (Cicer arietinum; CA) flour. Seven formulations were produced: a milk-protein control (Ctr) and formulations with 50% or 100% replacement by white lupin (LA50 and LA100), grass pea (LS50 and LS100) or chickpea (CA50 and CA100) flour. Proximate composition was assessed descriptively, whereas pH, CIELAB colour, emulsion stability, texture profile analysis (TPA), oscillatory rheology (storage modulus, G', at 1 Hz) and sensory attributes were analysed by one-way ANOVA, followed by Tukey's HSD or unequal-N HSD multiple comparisons ($p < 0.05$).

Protein ranged from 15.39% to 17.89% and fat from 17.43% to 21.58%; LA100 showed the highest moisture and fat contents and the lowest protein content. Formulation affected pH ($p < 0.001$), although values remained within a narrow range (6.06-6.22), and significantly influenced all colour parameters ($p < 0.001$). Lupin produced darker and redder pâtés, with LA100 showing the lowest L* (61.67) and highest a* (8.33), while chickpea increased yellowness and colour saturation; CA50 showed the highest L* (70.25), and CA100 the highest b* (16.75) and C* (17.72). Emulsion stability also differed: LS50 showed the highest total expressible fluid (1.21%) and expressible fat (0.01%), and CA50 showed the lowest total expressible fluid (0.32%). Hardness, resilience, springiness, gumminess and chewiness were significantly affected, while adhesiveness and cohesion remained unchanged. LA50 and LA100 were less hard than Ctr; LA100 had lower gumminess than LS100 and CA50; CA50 showed greater springiness than Ctr and greater chewiness than LA100; and LS50 showed greater resilience than CA50 and CA100.

Rheological measurements further differentiated the formulations. G' at 1 Hz ranged from 63.1 to 119.7 kPa, being lowest in LA100 and LS100 and highest in CA100 and CA50. LA100 and LS100 showed lower G' than LS50, Ctr, CA100 and CA50, whereas LA50 was intermediate and lower only than CA100 and CA50. Thus, the low G' of LA100 agreed with its reduced hardness, while the response of LS100 indicated that small-deformation viscoelasticity and TPA hardness captured complementary structural properties. No significant formulation effects were detected for any sensory attribute or overall liking ($p > 0.05$).

Replacing milk protein with legume flours was technologically feasible without detectable changes in sensory ratings. Lupin, particularly at 100% replacement, promoted a softer, darker, redder and less rigid matrix; chickpea increased yellowness and chroma, generated the highest G' values and, at 50% replacement, favoured fluid retention; and grass pea showed a level-dependent response, with greater fluid and fat release at 50% replacement but low G' at 100%. These results support the use of legume flours as milk-protein replacers in hybrid liver pâté, with flour type and replacement level selected according to the desired balance between appearance, spreadable texture and physical stability[2,3].

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Bioactive Compounds after Gastrointestinal Digestion and Colonic Fermentation: The Case of Brassicaceae Microgreens

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Brassicaceae microgreens are young vegetables increasingly promoted as sources of glucosinolates (GSLs), isothiocyanates (ITCs) and (poly)phenols (PPs)[1]. However, their potential biological relevance depends not only on their initial composition, but also on the fraction that remains bioaccessible or is transformed during gastrointestinal digestion (GID) and colonic fermentation.

Thus, this systematic review assessed the effects of in vitro gastrointestinal digestion (GID) and colonic fermentation on the bioaccessibility of GSLs, ITCs and PPs from Brassicaceae microgreens. Following PRISMA guidelines, from 75 records identified, ten studies reporting quantitative data were selected (Figure 1), comprising 15 microgreens. All studies were published in the last six years, underscoring the novelty of this research area. Most studies (8/10) employed standardized in vitro GID protocols (INFOGEST[2,3]) with some variations. Regarding the techniques used for extraction, methanol-water mixtures (70–80%) were the most employed extraction solvents for all compounds, often acidified with formic acid. Analytical techniques included spectrophotometric assays and various high-performance liquid chromatographic methods, although heterogeneity limited direct comparisons.

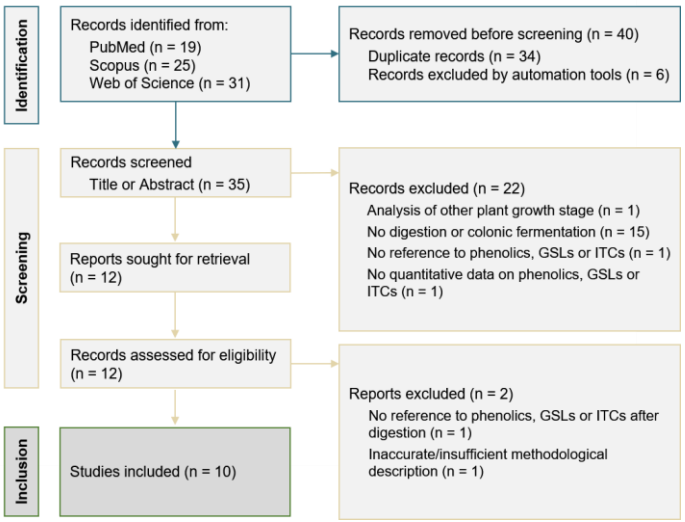


Figure 1 - Distribution PRISMA flow diagram.

GID and colonic fermentation markedly impacted compound bioaccessibility despite substantial variability (GSLs: not detected – 63.4% with newly formed ITCs; PPs: 4.1 – 67.9%), depending on species/variety/cultivar, food matrix, compound class and experimental conditions. Anthocyanins were consistently poorly bioaccessible (2.9 – 32.1%), while flavonoids and phenolic acids generally showed low-to-moderate recovery. Post-colonic fermentation bioaccessibility of total PPs ranged from 8.5 – 19.3%, with extensive transformation of parent compounds and formation of lower-molecular-weight catabolites, while evidence on GSLs and ITCs remained insufficient for quantitative synthesis. Thus, results consistently showed that raw composition does not reliably predict bioaccessibility, highlighting the importance of this type of studies. Critical gaps included limited simultaneous tracking of GSLs/ITCs conversion pathways, poor characterization of PP conversion pathways and scarce colonic fermentation studies. Overall, the evidence indicates that compositional data alone are insufficient to estimate the nutritional relevance of Brassicaceae microgreens, and that future research should combine harmonised, time-resolved digestion and fermentation protocols with comprehensive metabolite profiling using robust chromatographic quantification techniques. Complex multicellular models and well-designed human studies can also help clarify bioavailability, metabolism, and biological effects, thereby supporting the translation of in vitro evidence into dietary applications.

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Influence of processing conditions on the mechanical properties of high-moisture pea protein extrudates

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The increasing demand for plant-based meat analogues has stimulated the development of high-moisture extrusion (HME) as an effective technology to obtain fibrous protein structures with meat-like texture (Figure 1). Processing conditions, particularly barrel temperature and screw speed, play a decisive role in protein denaturation, molecular interactions, melt rheology and energy consumption, ultimately determining the structural and mechanical properties of the final product[1,2]. This study aimed to evaluate the effects of barrel temperature and screw speed on the mechanical properties and specific mechanical energy (SME) of high-moisture pea protein extrudates. Extrudates were produced using two final barrel temperatures (130 and 140 °C) and two screw speeds (200 and 400 rpm). The products were characterized in terms of fibrous degree, hardness, cohesiveness, springiness, force of rupture, tensile strength, deformation at break, puncture force and SME. Temperature significantly affected hardness ($p = 0.006$), force of rupture ($p < 0.001$), tensile strength ($p < 0.001$) and puncture force ($p < 0.001$), whereas screw speed alone had no significant effect on the evaluated mechanical properties. Significant temperature and screw speed interactions were observed for force of rupture, tensile strength and deformation at break. Increasing screw speed substantially increased SME, from 145.95 ± 9.91 and 125.65 ± 0.05 kJ kg^{-1} at 200 rpm to 299.82 ± 3.75 and 252.03 ± 0.06 kJ kg^{-1} at 400 rpm, which is consistent with the greater shear, mixing intensity, torque and residence time distribution generated during extrusion[2]. Conversely, increasing the processing temperature reduced SME while enhancing the mechanical resistance of the extrudates. This behavior suggests that higher processing temperatures promoted protein unfolding and favored stronger intermolecular interactions, leading to the development of a more cohesive protein network, in agreement with previous observations that increasing processing temperature enhances protein network formation during high-moisture extrusion[1]. Under the processing conditions evaluated, barrel temperature exerted a stronger influence on the mechanical properties of the extrudates than screw speed, indicating that thermal conditions play a more decisive role in determining the structure and texture of pea protein-based meat analogues.



Figure 1 - Pea protein extrudates and fiber formation at 140ºC and 200rpm.

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Development of Functional Herbal Infusions through the Upcycling of Fifth-Range Beetroot Surplus and Orange Juice Processing By-Products

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The valorization of agro-industrial by-products supports the development of sustainable functional beverages while reducing food waste. Beetroot granules (BG), obtained by low-temperature drying of pomace produced from fifth-range beetroot surplus after juice removal, are rich in betalains and antioxidant compounds. Orange granules (OG), produced from orange pomace generated during citrus juice processing, provide complementary phenolic compounds and organic acids that may improve betalain stability by lowering infusion pH[1,2]. However, the combined use of BG and OG in functional herbal infusions remains largely unexplored. Therefore, herbal infusions containing different BG:OG ratios (w/w) were evaluated for their phytochemical composition, antioxidant activity, interaction effects and short-term stability (Figure 1).

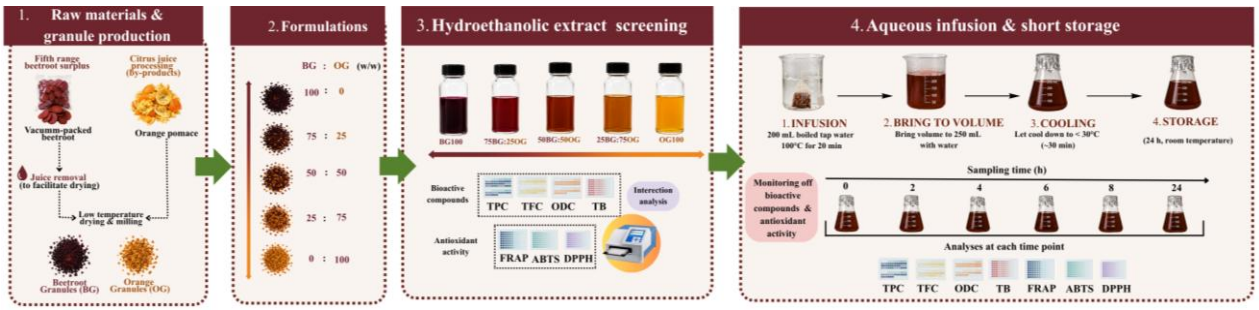


Figure 1 - Experimental workflow for the development and characterization of BG:OG herbal infusions.

Hydroethanolic screening highlighted the complementary phytochemical characteristics of BG and OG. While most BG: OG mixtures exhibited additive behavior, significant synergistic interactions for TPC, TFC and ODC were observed, particularly in the 75BG:25OG formulation, suggesting that combining both upcycled ingredients may enhance the recovery of specific phenolic fractions. In aqueous infusions, increasing OG incorporation enhanced phenolic content while progressively lowering infusion pH, whereas higher BG proportions promoted betalain content and antioxidant capacity. Among the blended formulations, 75BG:25OG exhibited the most balanced phytochemical profile, combining the highest antioxidant activity (FRAP: 63.22 ± 1.07, ABTS: 47.99 ± 1.25 and DPPH: 40.69 ± 2.51 µmol TE/100 mL) with 1.12 ± 0.04 mg/100 mL total betalains. During 24 h of storage, this formulation also showed the most consistent retention of phytochemicals and antioxidant activity, while total betalains remained stable. The distinct phytochemical characteristics of BG and OG resulted in formulations integrating phenolic compounds and betalains with enhanced antioxidant capacity, highlighting the potential of their combined use for functional herbal infusion development. Overall, these preliminary findings support the valorization of fifth-range beetroot surplus and citrus processing by-products as complementary ingredients for naturally functional herbal infusions, contributing to circular bioeconomy strategies and the sustainable use of agro-industrial side streams.

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Solvent-dependent recovery of bioactive compounds from *Pleurotus citrinopileatus* for functional food applications

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Pleurotus citrinopileatus is an edible mushroom with potential as a source of natural ingredients for functional foods and food-protection applications. This study compared a polysaccharide-rich aqueous extract and a phenolic-rich methanolic extract regarding their chemical composition, antioxidant capacity and antibacterial activity. Total phenolics, ortho-diphenols and flavonoids were determined by colorimetric methods, while antioxidant activity was evaluated using the ABTS assay. Antibacterial activity was assessed by disk diffusion on Mueller–Hinton agar at 1000 µg/mL against Pseudomonas aeruginosa, a bacterium relevant to food contamination, spoilage and persistence in food-processing environments.

The methanolic extract showed a higher total phenolic content than the aqueous extract, reaching 44.39 and 17.85 mg gallic acid equivalents/g dry weight, respectively, and approximately 1.9-fold greater ABTS radical-scavenging activity. Conversely, the aqueous extract showed a 3.2-fold higher response in the ortho-diphenol assay and exhibited the strongest antibacterial effect, outperforming the corresponding methanolic extract.

These results demonstrate that solvent selection produces chemically and functionally distinct fractions. The phenolic-rich methanolic extract was mainly associated with antioxidant activity, whereas the polysaccharide-rich aqueous extract showed greater antibacterial potential. This complementary functionality supports the valorisation of P. citrinopileatus as a source of tailored natural ingredients for functional food development and microbial control in food systems.

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The benefits of *Camellia sinensis* green tea and whey protein combination to improve human physical exercise and sports performance

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As the global population expands, the demand for dietary protein continues to rise and at present, consumption relies heavily on animal-derived sources, notably meat and dairy products. Among these, milk products, particularly whey proteins, are widely utilized in dietary supplements, nutrition bars, and sports supplement drinks, due to their high level of essential amino acids. Whey proteins have transitioned from being a polluting waste product into a multifunctional food ingredient due to its nutritional and functional properties.1 However, plants, like Camellia sinensis, are one of the most consumed beverages, renowned for its health benefits. These properties are largely driven by polyphenols, which are utilized in pharmaceuticals, nutraceuticals, cosmetics, and foods due to their antioxidant, anti-carcinogenic, antibacterial, anti-inflammatory, and anti-apoptotic effects.2 Combining plant and animal proteins is a promising strategy for enhancing both the nutritional value and functional properties of plant-based sources. Moreover, this blending approach creates innovative marketing opportunities for the food industry, driving the development of novel products with superior techno-functional and sensory attributes.3 This study aims to determine the antioxidant properties and protein content in different formulations with green tea and whey proteins for sports performance improving body function during physical exercise. Spectrophotometric assays were used for all analyses, and the results showed (Table 1) that increasing the green tea to whey ratio from 1:1 to 2:0.5 significantly enhanced total phenolic content (from 193.09 ± 1.96 to 261.89 ± 1.18 mg GAE/g DE), flavonoids, and tannins. The results also showed that antioxidant capacity improved drastically, as evidenced by the significant decrease in EC50 values for both free radical scavenging activity (from 13.05 ± 0.07 to 7.83 ± 0.04 µg/mL) and ferric reducing antioxidant power (from 15.46 ± 0.25 to 10.13 ± 0.05 µg/mL). This increased antioxidant efficiency is directly correlated with the higher concentration of hydroxyl groups from the tea polyphenols. Conversely, protein content dropped from 234.33 ± 4.93 to 119.33 ± 3.06 µg/mL, which is attributed to the dilution of the protein whey source and/or the formation of insoluble polyphenol-protein complexes. In conclusion, the 2:0.5 ratio maximizes the functional and antioxidant potential of the formulation, while the 1:1 ratio increases the protein content. Furthermore, the consumption of antioxidants, such as green tea polyphenols, improves the body's antioxidant status by reducing the damage effects of free radicals during physical exercise, while the whey contains all essential amino acids that the human body cannot synthesize on its own.

Table 1 – Antioxidant activities (FRSA - free radical scavenging activity, FRAP - ferric reducing antioxidant power), TPC - total phenolic content, TPC - total flavonoid content) and tannins content in different formulations with green tea from *Camellia sinensis* and whey.

	Green tea to whey ratio		
	1:1	2:1	2:05
FRSA (EC_{50}, µg/mL)	13.05 ± 0.07	9.50 ± 0.08	7.83 ± 0.04
FRAP (EC_{50}, µg/mL)	15.46 ± 0.25	11.40 ± 0.02	10.13 ± 0.05
TPC (mg GAE/g DE)	193.09 ± 1.96	229.19 ± 0.65	261.89 ± 1.18
TFC (mg RE/g DE)	36.42 ± 0.20	39.95 ± 0.42	46.55 ± 0.42
Tannins (mg TAE/g DE)	160.05 ± 0.32	197.79 ± 0.78	218.00 ± 0.56
Protein (µg/mL)	234.33 ± 4.93	192.67 ± 2.31	119.33 ± 3.06

[a]Values are mean ± SD (*n=3*); EC_{50}, half-maximal effective concentration; GAE, gallic acid equivalents; RE, rutin equivalents; TAE, tannic acid equivalents; DE, dry extract.

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Influence of drying method on anthocyanins, phenolic compounds and antioxidant capacity of raspberry and blackberry powders

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Raspberries (Rubus idaeus L.) and blackberries (Rubus fruticosus L.) are highly perishable fruits, and part of their production may be excluded from the fresh market due to visual imperfections related to size, shape, or colour, despite retaining considerable nutritional and bioactive value[1,2]. Transforming these fruits into powder may represent a sustainable strategy to reduce food losses and obtain value-added ingredients rich in bioactive compounds[2]. During powder production, the drying method may significantly affect the preservation of heat-sensitive compounds, particularly anthocyanins[3]. This study evaluated the effects of two drying methods, freeze-drying and hot-air drying, on the physicochemical characteristics, phenolic composition, and antioxidant capacity of raspberry and blackberry powders.

Raspberry and blackberry batches were divided into two portions and either freeze-dried at 0.2 hPa using a ScanVac CoolSafe Freeze Dryer, with the condenser temperature set to −49 °C, or hot-air dried at 50 °C for 48 h. After drying, the samples were milled and sieved to reduce the seed fraction and particle size, thereby obtaining finer, more homogeneous powder fractions. The fresh fruits and the resulting powders were characterised for moisture content, colour, total phenolic content, total anthocyanin content, and antioxidant capacity using the DPPH and FRAP assays.

Fresh raspberries exhibited a total anthocyanin content of 173.35 ± 3.61 mg cyanidin-3-glucoside equivalents/100 g DW. Freeze-drying retained 94% of the initial anthocyanin content, resulting in 163.28 ± 3.07 mg/100 g DW, whereas hot-air drying retained only 39%, with a final content of 67.71 ± 1.91 mg/100 g DW. A similar pattern was observed for blackberries, although anthocyanin retention was lower after freeze-drying than in raspberries. Freeze-dried blackberry powder retained 79% of the anthocyanins present in the fresh fruit, while hot-air-dried powder retained 46%, corresponding to 277.41 ± 1.87 and 161.38 ± 4.21 mg/100 g DW, respectively. These results indicate that anthocyanins were particularly susceptible to the prolonged thermal exposure associated with hot-air drying.

Both drying methods also caused marked changes in colour relative to the corresponding fresh fruits. For raspberry powders, ΔE∗ab values were 15.80 after freeze-drying and 15.53 after hot-air drying, whereas values of 22.04 and 20.01 were obtained for freeze-dried and hot-air-dried blackberry powders, respectively. In all cases, the colour differences were mainly associated with an increase in lightness. The blackberry powders additionally exhibited pronounced increases in the red component, particularly after freeze-drying. Therefore, although freeze-drying provided better anthocyanin retention, it did not result in lower overall colour differences relative to the fresh fruits.

Total phenolic content was 383.69 ± 6.50 mg gallic acid equivalents/100 g DW in freeze-dried raspberry powder and 393.37 ± 10.64 mg/100 g DW in hot-air-dried raspberry powder. For blackberry powders, the corresponding values were 416.38 ± 3.72 and 410.22 ± 6.07 mg/100 g DW, respectively. Overall, total phenolic contents were relatively similar between drying methods, suggesting that drying had a smaller effect on total phenolics than on anthocyanins.

Nevertheless, freeze-dried powders consistently exhibited greater antioxidant capacity than their hot-air-dried counterparts. Freeze-dried raspberry powder showed the highest DPPH value, reaching 5489.92 ± 46.35 µmol Trolox equivalents/100 g DW, while freeze-dried blackberry powder exhibited the highest FRAP value, at 67.15 ± 2.28 mmol Fe^{2+} equivalents/100 g DW. The simultaneous reduction in anthocyanin content and antioxidant capacity after hot-air drying suggests that anthocyanin degradation may have contributed to the loss of antioxidant capacity, although this relationship should be confirmed through correlation analysis.

Overall, both drying technologies enabled the production of berry powders containing relevant levels of bioactive compounds. However, freeze-drying preserved a substantially greater proportion of the initial anthocyanins and resulted in higher antioxidant capacity than hot-air drying. By contrast, total phenolic contents were comparatively stable among the powders, and freeze-drying did not minimise the overall colour difference relative to the fresh fruits. These findings indicate that freeze-drying is the preferred method when the preservation of anthocyanins and antioxidant properties is the primary objective, supporting the valorisation of non-marketable raspberries and blackberries as bioactive food ingredients.

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Comparative Chemical Profiling, Antimicrobial Activity, and Cytotoxicity of *Thymus mastichina* and *Helichrysum italicum* Essential Oils

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Thymus mastichina L. and *Helichrysum italicum* (everlasting flowers) are aromatic species belonging to the *Lamiaceae* and *Asteraceae* families, respectively. Both plants are traditionally recognised as medicinal, ornamental, and edible species and are native to Europe, the Mediterranean region, and the western Himalaya1. Their occurrence within the biodiversity of Serra da Estrela highlights their value as local bioresources with promising potential for the sustainable development of health-promoting natural products[1,2].

In the present study (Figure 1), essential oils (EOs) obtained from the aerial parts of *T. mastichina* and *H. italicum* were investigated using an integrated chemistry-to-biology approach. The study included EO characterisation by GC–MS, antimicrobial evaluation through the determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against Gram-positive and Gram-negative bacteria using the broth microdilution method, according to the Clinical and Laboratory Standards Institute guidelines[3], and cytotoxicity assessment in normal human dermal fibroblasts (NHDF) and hepatocellular carcinoma cell line (HepG2) using MTT and LDH assays.

GC–MS analysis showed that *T. mastichina* essential oil was dominated by oxygenated monoterpenes and monoterpene hydrocarbons, with 1,8-cineole, p-cymene, camphor, thymol, γ-terpinene, borneol, camphene, α-pinene, β-pinene, and linalool as the principal constituents. In contrast, H. italicum essential oil was mainly characterised by α-pinene, epi-α-bisabolol, trans-caryophyllene, α-copaene, α-humulene, and β-caryophyllene. The predominant constituent of T. mastichina EO, 1,8-cineole, has previously been reported to possess cytotoxic and antitumour activity against HepG2 cells[4], as well as anti-inflammatory, antibacterial, and antifungal properties[5]. Similarly, α-pinene, one of the main constituents of H. italicum EO, has been reported to exhibit cytotoxic, antimicrobial, and antitumour activities[6].

Both EOs exhibited noteworthy antibacterial and antifungal activities against the tested microbial strains, indicating their potential as natural antimicrobial agents. However, potential antagonistic interactions should be considered when combining these EOs, as they may reduce their biological efficacy. Further studies are therefore required to clarify their mechanisms of action and potential interactions.

Cytotoxicity assays demonstrated that NHDF fibroblasts maintained high viability at lower EO concentrations, whereas LDH release increased at higher concentrations, indicating concentration-dependent membrane damage. In HepG2 cells, both essential oils induced a cytotoxic effect, characterised by reduced cell viability and increased cellular damage, particularly at higher concentrations. These findings indicate that the biological effects of the EOs are concentration- and cell-type-dependent and suggest that HepG2 cells may be more sensitive to EO exposure.

The chemical profile of both essential oils is consistent with their antimicrobial and cytotoxic activities, supporting the potential of T. mastichina and H. italicum as promising sources of bioactive compounds for pharmaceutical and nutraceutical applications. However, further studies are required to establish their mechanisms of action and define safe and effective concentration ranges.

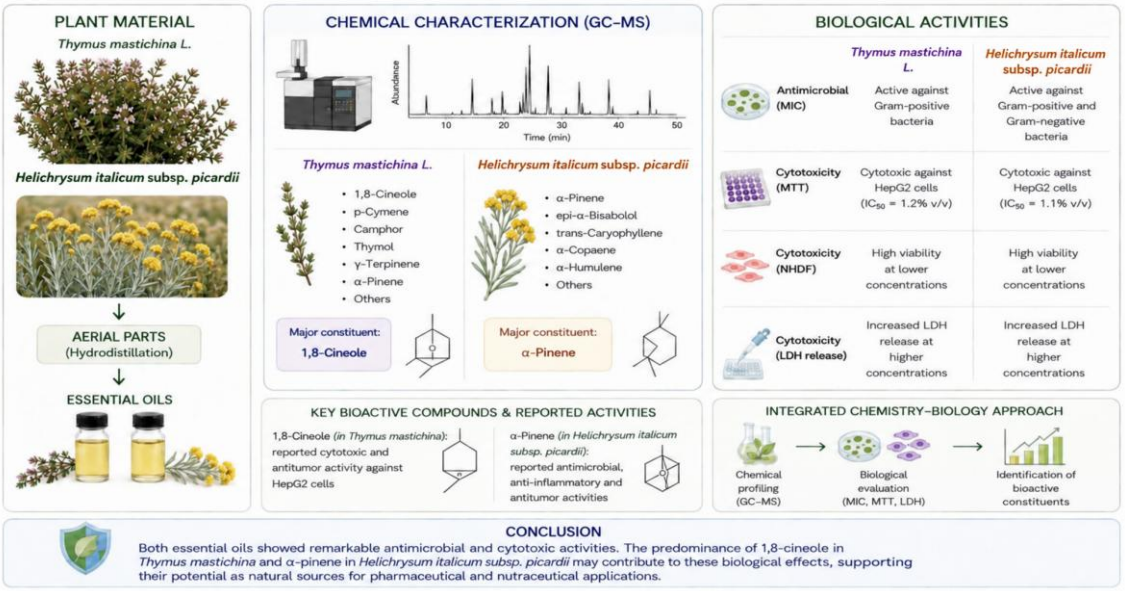


Figure 1 - Comparative chemical profiling, antimicrobial activity, and cytotoxicity of *Thymus mastichina* and *Helichrysum italicum* essential oils.

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Impact of Pulse Flour Incorporation on the Quality Profile of Hybrid Cooked Hams

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The growing interest in hybrid meat products as a strategy for more sustainable food production needs a thorough understanding of how plant-based ingredients affect the technological properties of familiar meat items. This study evaluated the impact of replacing meat with chickpea and lupin flours at 0 (control), 5, and 10% incorporation levels on the physicochemical and textural quality of cooked ham. Analyses included pH, water activity (aW), surface and internal colour (CIELab, chroma, hue), and texture profile analysis (hardness, cohesiveness, springiness, gumminess, chewiness). Data were analysed using factorial ANOVA and Tukey's test ($p<0.05$).

The results revealed that both flour type and incorporation level significantly influenced product quality. Lupin flour caused a significant reduction in pH at both levels, while chickpea flour had no significant effect. Water activity increased with chickpea addition but significantly decreased with lupin, particularly at the 10% level. Colour was markedly altered by both flours, primarily through increased yellowness and hue angle, with the most pronounced effect observed for lupin at 10%, consistent with findings reported for other pulse-based hybrid systems[1]. The most discriminating parameter was texture. Chickpea formulations showed significantly lower hardness, gumminess, and chewiness compared to the control but retained a structure closer to that of the conventional product, although with higher variability, suggesting less uniform matrix formation. In contrast, lupin incorporation led to a more pronounced weakening of the product matrix, as indicated by significant decreases in hardness, cohesiveness, springiness, gumminess, and chewiness, corroborating observations that flour type critically determines structural integrity in hybrid meats[2].

In conclusion, the feasibility of producing hybrid cooked ham is highly dependent on the pulse type and its inclusion level. Based on the parameters evaluated, 5% chickpea flour incorporation demonstrated the most balanced technological performance, positioning it as a promising option for developing hybrid cooked ham with a quality profile similar to the conventional product. These findings contribute to the growing body of knowledge on hybrid product development, highlighting the importance of ingredient selection and formulation optimization.

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Valorising *Sanguisorba minor* from Serra da Estrela: Anti-inflammatory Activity, Cancer-Relevant Bioassays and Perspectives for Human Well-Being

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Sanguisorba minor Scop., commonly known as salad burnet, is a perennial species belonging to the Rosaceae family. Native to Europe, the Mediterranean region, and the western Himalaya, this edible and medicinal plant is an interesting botanical resource for developing natural health-promoting products[1]. Its presence in the biodiversity of Serra da Estrela further supports its local valorisation within a sustainable bioresource-based approach[2,3,4]. In this study, an infusion prepared from the flowers/leaves of *S. minor* was evaluated through an integrated chemistry-to-biology approach, including extraction yield, total phenolic content, antioxidant activity, α-glucosidase inhibition, cytocompatibility and anti-inflammatory assessment. The infusion showed an extraction yield of 12.88 ±0.21% and a high total phenolic content of 336.393 ± 10.222 mg GAE/g DW, suggesting a rich phenolic profile. It exhibited relevant antioxidant activity, particularly against superoxide radicals, with an IC_{50} of 9.263 ± 0.476 µg/mL, outperforming ascorbic acid in this assay. The infusion also showed $DPPH^{\bullet}$ and $NO^{\bullet}$ scavenging activities, with IC_{50} values of 12.939 ± 0.913 µg/mL and 66.944 ± 1.344 µg/mL, respectively. Notably, *S. minor* displayed strong α-glucosidase inhibitory activity, with an IC_{50} of 3.119 ± 0.0761 µg/mL, which was markedly lower than that of acarbose, used as the positive control (IC_{50} = 148.876 ± 21.501 µg/mL). This result highlights the potential relevance of this infusion to modulating postprandial carbohydrate digestion. The cellular assays provided an important biological interpretation of these chemical activities. In NHDF fibroblasts, MTT values remained close to the control at most concentrations, whereas LDH leakage increased markedly at higher concentrations, especially from 400 to 800 µg/mL, indicating membrane damage at elevated doses. In HepG2 cells, used here as a cancer-relevant cellular model, the infusion induced a time- and concentration-dependent decrease in metabolic activity, accompanied by increased LDH release, particularly after 48–72 h and at the highest concentrations. In RAW 264.7 macrophages, cytotoxicity was also concentration-dependent, with stronger effects at 400–800 µg/mL. Importantly, in LPS-stimulated RAW 264.7 cells, S. minor reduced nitric oxide production compared with the LPS control, supporting a promising anti-inflammatory effect, especially when interpreted within the non- or low-cytotoxic concentration range. These findings indicate that *S. minor* from Serra da Estrela is a phenolic-rich infusion with antioxidant, α-glucosidase-inhibitory, and anti-inflammatory potential. The results also support its preliminary exploration in cancer-relevant bioassays, contributing to the valorisation of local plant biodiversity for human well-being.

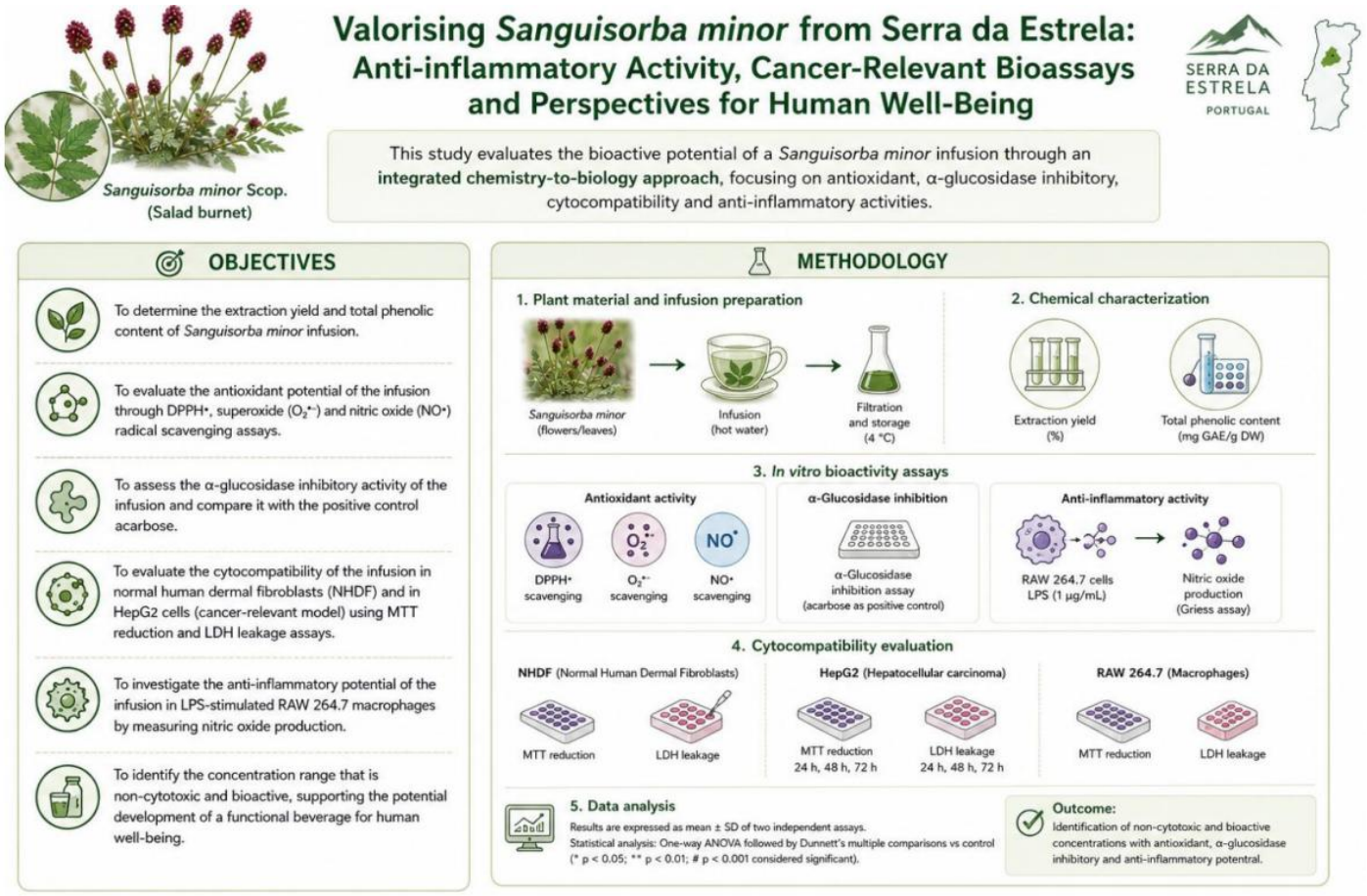


Figure 1 - Integrated Objectives and Methodology for the Bioactivity Assessment of Sanguisorba minor Infusion

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Prebiotic potential of soluble dietary fiber from apple juice after in vitro human fecal fermentation

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The increasing interest in functional foods has driven research into the potential of natural dietary fibers as prebiotic agents, able to nourish beneficial host microorganisms, namely the ones present in apple[1]. This study aimed to evaluate the prebiotic potential of two apple juices produced by SUMOL COMPAL through in vitro human fecal fermentation and comprehensive microbiota analysis.

Before fermentation the juices were characterized for their dietary fiber content by K-RINTDF from Megazyme. Juice A had 0.23% on fresh weight basis (%fw) of soluble dietary fiber (SDF) and 0.09%fw of insoluble dietary fiber (IDF) summing 0.32%fw total dietary fiber (TDF) with a ratio of SDF/IDF of 2.5. On the other hand, juice B had higher amount of both SDF (0.64%fw) and IDF (0.55%fw), summing 1.20%fw of TDF, but a lower ratio SDF/IDF (1.2). For fermentation the high molecular weight material (HMWM) of both juices was isolated by dialysis (1 kDa cut-off) and juice B HMWM was further filtrated to evaluate the fermentative potential of soluble material alone. The HMWM of juices A and B contained 57 and 63 % of total sugars and were mainly composed by Ara, Gal and uronic acids (67 and 63 mol%), indicating that these fibers were mainly composed by pectic polysaccharides, followed by Glc (19 and 23 mol%) and lower amounts of Xyl, Rha, and Fuc. The isolated soluble material had a similar amount of total sugars (65%) but a higher amount of pectic polysaccharides (71 mol%).

After in vitro human fecal fermentation of the three samples from apple juices, the fermentation supernatants were assessed for carbohydrate profile by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and short-chain fatty acid (SCFA) by high-performance liquid chromatography (HPLC). The fermentation pellets were used to investigate the microbiota through 16S rRNA gene sequencing followed by alpha- and beta-diversity analyses.

Carbohydrate profile indicated polysaccharide depolymerization during the first 12 hours with all samples, followed by microbial utilization of the released oligosaccharides and monosaccharides after 24 and 48 h. HMWM from juice A and the soluble HMWM of juice B showed higher amounts of SCFA, especially acetate and propionate, and increased the relative abundance of beneficial bacteria, such as Bifidobacterium bifidum and Blautia faecis. Alpha- and Beta-diversity metrics further supported the positive modulation of microbial communities, as apple fibers exhibited effects comparable to those of inulin, a well-established prebiotic. These findings highlight the prebiotic potential of apple juice dietary fiber and support the importance of implementation of technological strategies to increase its content, particularly that of soluble dietary fiber, in apple juices.

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Extraction and Analytical Mapping of Biocompounds from *Ruscus aculeatus* L. Pulp : Influence of Supercritical Fluid Modifiers on Functional Profile

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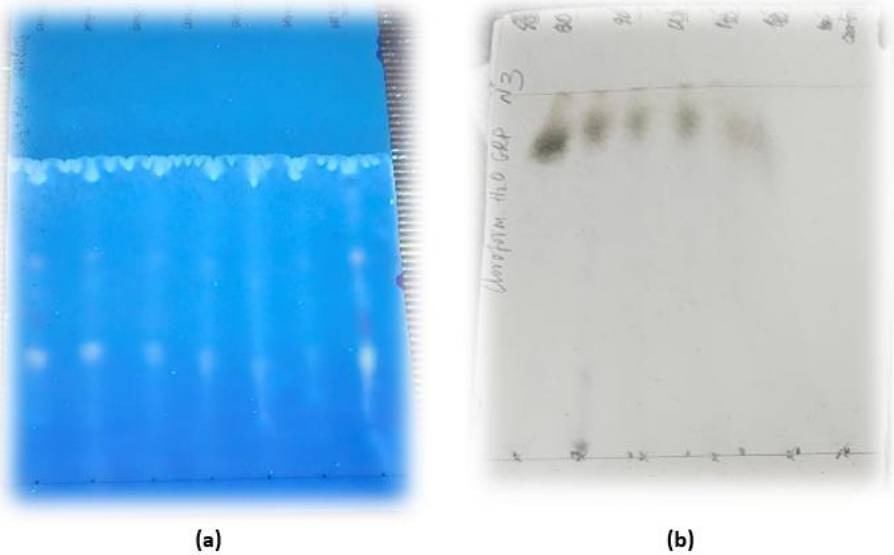
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The search for new bioactive compounds from wild matrices has driven food chemistry towards more sustainable processes. *Ruscus aculeatus* (butcher's broom) is an evergreen shrub with a long history of use in traditional medicine due to its anti-inflammatory, venotonic, and vascular protective properties, attributed to its richness in bioactive compounds, particularly steroidal saponins, flavonoids, and phenolic acids[1]. Supercritical fluid extraction using carbon dioxide (SCFE) represents a sustainable alternative for obtaining these compounds while avoiding organic solvent residues[2].

This work aimed to evaluate the influence of $scCO_2$ modifiers on the chemical profile of Ruscus aculeatus pulp extracts while developing a rapid micro-scale analytical workflow for their preliminary characterization. Extractions were performed using pure CO_2 and CO_2 modified with water or ethanol under different operating conditions. The resulting extracts were analyzed by complementary thin-layer chromatography (TLC) employing three orthogonal mobile phases targeting compounds of different polarity, e.g. system S_2, toluene: ethyl acetate and chloroform: methanol: water. Chromatographic plates were visualized under UV light at 366 nm and with derivatization through selective reagents.

The chromatographic fingerprints clearly demonstrated that the supercritical fluid modifier strongly influences the composition of the recovered fractions. Extraction with pure CO_2, produced predominantly low-polarity profiles, observed in the chloroform system, as it could be phytosterols, free fatty acids, and low-polarity terpenes, whereas the addition of water significantly enhanced the resolution of polar constituents, with fluorescence patterns compatible with flavonoid-related compounds (Figure 1, (a)). Conversely, other less-polar compounds were also well-resolved probably indicating the presence of saponin, lipophilic sterols, proanthocyanidins, and monoterpene-based exemplars (Figure 1, (b)).



(a) (b)

Figure 1 – TLC of extracts obtained by SCFE using CO_2+H_2O binary co-solvent system: (a) in S_2-based system; and (b) chloroform-based system.

Ethanol-assisted SCFE generated the broadest chromatographic profile, both in S_2 and chloroform systems, revealing improved recovery of both medium- and low-polarity constituents, e.g. coumarins and free phenolic acids, and including bands compatible with steroidal saponins, such as ruscogenin and neoruscogenin, alongside medium-polarity triterpenes[3]. Overall, this study demonstrates that SCFE assisted by polar co-solvents, particularly ethanol and water, enhances the recovery of polar bioactive fractions from Ruscus aculeatus pulp compared with pure CO_2. Furthermore, the combination of complementary TLC elution systems provides a rapid, inexpensive and sustainable micro-scale workflow for the preliminary characterization and comparison of extracts obtained under different extraction conditions, while minimizing solvent and sample consumption prior to detailed chromatographic or spectrometric analyses. The resulting extracts,

enriched in natural antioxidants and other phytochemicals, represent promising candidates for future applications as bioactive ingredients in functional foods, nutraceuticals and other food-related products.

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Hot-water extraction and glucan profiling of *Agaricus bisporus* for in vitro prebiotic applications

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Edible mushrooms are recognized as valuable sources of structurally diverse polysaccharides, particularly β-glucans, which have attracted increasing interest as functional food ingredients with potential effects on the gut microbiota[1–3]. However, the development of microbiota-oriented ingredients requires reproducible extraction procedures and an accurate assessment of the glucan profile of the starting biomass and recovered fractions[1,2]. This study aimed to characterize the glucan content of selected edible mushroom species and to establish reproducible hot-water extraction conditions for *Agaricus bisporus* as a basis for subsequent in vitro evaluation of prebiotic potential

Total and α-glucans were enzymatically determined in *Pleurotus sp.*, *A. bisporus*, *Boletus sp*. and *Cantharellus cibarius*, while β-glucan content was estimated by difference. *A. bisporus* biomass was subsequently subjected to hot-water extraction under different solid-to-liquid ratios (1:20, 1:25, 1:30 and 1:35), temperatures (60, 70, 80 and 90 °C) and extraction times (30, 60 and 120 min). Each condition was evaluated in triplicate, and crude extraction yield was determined gravimetrically. The extraction condition was selected considering mean yield, processing time and experimental reproducibility.

Among the species evaluated, *Pleurotus sp*. presented the highest estimated β-glucan content, approximately 33.87%. *A. bisporus* contained 19.49% total glucans, 0.45% α-glucans and 19.04% estimated β-glucans, indicating that β-glucans represented approximately 97.7% of its total glucan fraction. For *A. bisporus*, increasing the extraction temperature from 60 to 90 °C increased the mean crude extraction yield from approximately 6.88% to 9.28%. At 90 °C for 60 min, solid-to-liquid ratios of 1:20 and 1:30 generated comparable mean yields, approximately 9.28% and 9.30%, respectively. However, the 1:20 ratio showed markedly greater reproducibility, with a coefficient of variation of approximately 0.40%, compared with approximately 14.0% for the 1:30 ratio. At a 1:20 ratio and 90 °C, extraction for 30, 60 and 120 min produced mean yields of approximately 8.84%, 9.28% and 8.60%, respectively.

Based on crude extraction yield, processing time and reproducibility, the combination of a 1:20 solid-to-liquid ratio, 90 °C and 60 min was selected for downstream analysis. This condition provides a robust basis for the subsequent determination of β-glucan content in the recovered extract and for in vitro prebiotic screening using selected beneficial intestinal bacteria. The results support A. bisporus as a promising source of mushroom-derived glucans and establish the technological foundation for the development of functional ingredients intended for gut microbiota-oriented applications. Further studies will assess the ability of the selected extract to support beneficial bacterial growth and investigate the relationship between glucan composition and prebiotic response.

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Potato Protein-Based Meat Analogues Fortified with Upcycled Fruit Flours: Functional, Textural, and Antioxidant Insights

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The rise of healthy lifestyle trends and vegetarian/vegan food regimes has driven significant food innovation, with a focus on developing and improving plant-based products, such as emulsion-based products. However, replacing animal-based protein with a vegan analogue is a complex process that requires extensive research to ensure a nutritionally and texturally satisfying product[1]. Egg white protein (EWP) is widely used in the food industry, due to its excellent emulsifying, gelling, and water-binding properties[2]; however, the food industry is looking for alternatives to EWP, considering its limitations, including allergenicity, elevated cholesterol levels, incompatibility with vegan and plant-based formulations, price volatility, and sustainability concerns associated with animal-derived ingredientes[3].
This study aims to evaluate potato protein (PP) as a substitute for egg white protein (EWP) in vegan formulations. Fruit flours, including orange peel, pineapple peel, and pineapple core, were incorporated to assess their effects on the techno-functional and textural properties, and on the antioxidant potential of plant-based products. Fruit flours improved the performance of PP-based emulsions. More negative zeta potential values and an increased emulsifying stability index were observed in flour-containing formulations. A significant increase in the hardness parameter of the PP-based formulations containing fruit flour was observed, correlated with the higher water-holding capacity. Although fruit flours increased the phenolic content, the enhancement of antioxidant activity was dependent on the fruit flour. Colorimetric analysis showed protein source- and flour-dependent effects, with lower CIELAB values in PP-based samples. Overall, this work highlights the potential of PP and fruit flours for developing functional and sustainable plant-based food products.

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Assessing Lupin Flour Antioxidant Potential as a Sustainable Functional Ingredient in the Pastry Industry

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Introduction: As the global food system transitions toward sustainable and low-impact plant-based resources, legumes are being re-evaluated as high-performance functional ingredients. This study explores the antioxidant potential of lupin (Lupinus spp.) flour, a promising clean-label ingredient with potential interest for the development of alternative food matrices in the pastry industry[1,2]. To comprehensively conclude on its antioxidant power, the extracts are evaluated through the determination of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and free radical scavenging activity (DPPH radical assay).

Methods: Phenolic compounds were extracted from ground seed samples using various solvents (H_2O and ethanol concentrations at 25%, 50%, 80%, and 100% v/v), solid-to-solvent ratios (1:20, 1:50, and 1:100), and extraction times (30 min, 1 h, and 2 h) under constant agitation at room temperature. TPC was quantified spectrophotometrically at 765 nm using the Folin-Ciocalteu colorimetric method based on the reduction of a phosphomolybdate-phosphotungstate complex, with results expressed in mg gallic acid equivalents (GAE)/100 g DW. TFC was determined at 420 nm via a modified colorimetric assay using aluminum chloride to facilitate complex formation, with results reported in mg quercetin equivalents (QE)/100 g DW. Free radical scavenging activity was assessed by measuring the absorbance reduction of a methanolic DPPH solution at 517 nm, and the antioxidant capacity was calibrated against a Trolox standard curve to calculate the Trolox Equivalent Antioxidant Capacity (TEAC) in mg Trolox/100 g DW[3]. All analytical procedures were performed in triplicate to ensure accuracy and reproducibility.

Results: The results revealed that aqueous extracts (H_2O) generally maximized bioactive extraction and antioxidant activity. The highest TPC was achieved under the conditions H_2O; 1:50; 2h (8.712 ± 0.508 mg GAE/100 g DW), followed closely by H_2O; 1:20; 2h (7.499 ± 0.527 mg GAE/100 g DW). Regarding TFC, the highest content was recorded in H_2O;1:20; 2h (0.850 ± 0.235 mg QE/100 g DW), while among the alcoholic fractions, EtOH 80%; 1:100; 2h demonstrated the best performance (0.368 ± 0.025 mg QE/100 g DW). In the DPPH assay, the maximum antioxidant capacity was obtained under H_2O; 1:100; 1h (0.933± 0.164 mg Trolox/100 g DW), showing similar stability at 2 h (0.921 ± 0.104 mg Trolox/100 g DW). Extracts obtained with EtOH 100% demonstrated the lowest efficiency across all evaluated parameters.

Conclusion: These trials demonstrate that optimizing parameters such as solvent, ratio, and extraction time is crucial to maximize the recovery of lupin bioactive compounds. Specifically, pure aqueous extractions combined with longer processing times (1 to 2 hours) established the optimal conditions for obtaining the highest yields of phenolics, flavonoids, and overall radical scavenging capacity. Water enhances the solubility and extraction of a broader range of phenolic compounds while promoting better penetration of the solvent into the food matrix. Conversely, absolute ethanol (EtOH 100%) proved to be the least efficient system, failing to effectively extract these target hydrophilic components. Validating these optimal processing parameters supports the integration of lupin flour as a functional ingredient into the pastry industry, driving the development of clean-label baking systems.

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Effect of Thermal Processing and Fraction Separation on the Proximate Composition of *Lupinus albus* var. *rumbo* Derivatives

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Lupinus albus L. is an attractive plant-based raw material owing to its high protein and lipid contents[1]. However, the development of lupin-derived ingredients requires a thorough understanding of how processing operations influence nutrient partitioning and chemical composition.

The aim of this study was to evaluate the effect of two thermal processing strategies and dehulling on the proximate composition and nutrient distribution of sweet lupin (*Lupinus albus var. rumbo*) derivatives.

Two processing procedures were compared: procedure A, consisting of soaking, cooking, five-day water immersion, and freeze-drying; and procedure B, consisting of soaking, cooking, direct blending with the cooking water, and freeze-drying. Each procedure was applied to both whole and dehulled seeds. Moisture, ash, crude fiber, crude protein, total fat, and total carbohydrates were determined in triplicate for freeze-dried pastes, isolated fractions (hulls and cooking water) and commercial reference ingredients.

Freeze-drying effectively reduced moisture from approximately 84% to below 3%, producing chemically stable powders suitable for storage. The freeze-dried hull contained 55.6 ± 0.88% crude fiber, confirming that the tegument represents the primary dietary fiber fraction. Accordingly, whole-seed pastes exhibited substantially higher crude fiber contents (16.6 ± 0.49% and 14.4 ± 0.84% for Procedures A and B, respectively) than dehulled pastes (4.34 ± 0.12% and 2.78 ± 0.09%). The commercial protein isolate contained 82.6 ± 0.35% crude protein. Procedure B retained 42.6 ± 0.37% crude protein in the freeze-dried paste, whereas the corresponding cooking water contained 20.7 ± 0.56% crude protein, demonstrating the partitioning of water-soluble proteins and low-molecular-weight peptides during thermal processing. Dehulling also increased the apparent lipid concentration to approximately 12.6% by removing the fiber-rich hull fraction. Total carbohydrates remained the predominant component of all freeze-dried pastes, ranging from 55.7% to 65.8%.

Thermal processing and fraction separation significantly affected the chemical composition and nutrient distribution of lupin derivatives. The substantial protein content recovered in the cooking water highlights its potential for by-product valorization, while dehulling provides an effective strategy for modulating the fiber-to-lipid ratio of the resulting ingredients. These findings contribute to the development of lupin-derived functional ingredients and provide valuable scientific knowledge to support these ingredients as egg substitutes in pastry products, contributing to the development of innovative and functional bakery formulations.

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NADES-Assisted Recovery of Grape Pomace Anthocyanins for Sustainable Postharvest Preservation

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Winery by-products, rich in anthocyanins, represent promising alternatives to synthetic colorants. Natural deep eutectic solvents (NADES) have emerged as sustainable extraction media due to their low toxicity, biodegradability, and ability to enhance the extraction and stabilization of bioactive compounds1. Within the NOVAPACK project, Portuguese grape residues were investigated as renewable sources of bioactive compounds for sustainable postharvest applications.
Bioactive compounds were recovered from Vinhão grape pomace (Vitis vinifera) using a choline chloride:lactic acid NADES (ChCl:LA, 1:2). Different biomass loadings (1, 5 and 10%, w/v) were evaluated, with 5% (w/v) loading providing the highest total phenolic content, outperforming conventional acidified methanol extraction. Bioactivity assays (ABTS, DPPH and FRAP) confirmed the high antioxidant capacity of the extracts. HPLC-DAD identified malvidin-3-O-glucoside as the predominant anthocyanin, showing up to 60% higher recovery than the conventional extraction method. FTIR-ATR and rheological analyses confirmed the successful formation of the NADES system and its shear-thinning behavior, supporting its suitability as a green extraction medium[1].
The optimized extract was incorporated into pomegranate pectin-based edible coatings and applied to fresh blueberries during refrigerated storage. The active coatings reduced cumulative weight loss, delayed fungal development, and better-preserved fruit quality compared to the uncoated control, demonstrating the potential of grape pomace anthocyanins as functional ingredients for sustainable postharvest preservation[2].
These findings highlight the potential of combining green extraction technologies and edible coatings to valorize winery by-products and promote more sustainable postharvest preservation strategies.



Figure 1 - Methodological flowchart of the experimental design: from green extraction to postharvest monitoring.

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Natural Bioactive Compounds as Multifunctional Tools for Animal Derived Food and Feed Safety

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The growing demand for safe, sustainable, and high-quality animal derived products has driven increasing interest in natural bioactive compounds for applications across the food and feed chain. Polyphenols, essential oils, plant extracts, organic acids, antimicrobial peptides, bacteriocins, chitosan and other polysaccharides, enzymes, and natural antioxidants of animal origin have emerged as multifunctional alternatives to synthetic additives and antibiotics[1].

In animal production, compounds such as quercetin, rutin, resveratrol, thymol, and carvacrol have shown protective effects against mycotoxin induced hepatotoxicity, improved gut microbiota, and enhanced growth performance, egg and meat quality, while tannin rich feeds contribute to reducing methane emissions in ruminants[2]. In parallel, these compounds play an important role in food preservation, controlling foodborne pathogens such as Listeria monocytogenes, Salmonella spp., and Escherichia coli, and reducing oxidative deterioration in meat, dairy, fish, and egg products. Organic acids, lysozyme, the lactoperoxidase system, and antimicrobial peptides such as nisin and pediocin have been effective, particularly when combined with non-thermal technologies such as high-pressure processing, for a more natural preservation of meat products. Particular attention is given to their incorporation into active and intelligent food packaging, including whey protein and chitosan-based films enriched with essential oils, plant extracts, or anthocyanins, which combine antimicrobial and antioxidant protection with real time freshness monitoring. Many of these bioactives are recovered from agro-industrial by-products, such as grape pomace, olive leaves, pomegranate peel, and crustacean shells, supporting circular economy strategies and sustainable resource valorization[3].

Despite this potential, challenges remain regarding compound stability, bioavailability, sensory impact, standardization, and regulatory approval within the European Union, which must be addressed to enable wider industrial application. Overall, this work provides an integrated overview of key bioactive molecules and their strategic relevance for simultaneously improving feed efficiency, animal health, and the safety and quality of animal derived foods, aligned with the One Health concept and current consumer demand for natural and clean label solutions.

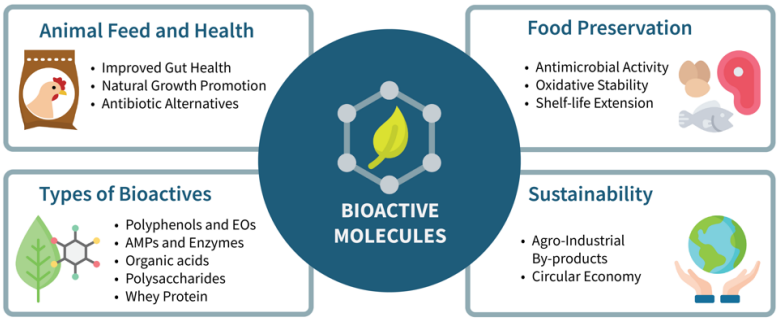


Figure 1 - Natural Bioactive Compounds as Multifunctional Tools for Animal Derived Food and Feed Safety.

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In silico identification of multifunctional peptides from EU-authorised edible insects with potential activity against hypertension, diabetes and Alzheimer's disease

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Sustainable food systems have increasingly highlighted edible insects as promising sources of nutrients and bioactive compounds.
The recent authorisation of *Acheta domesticus*, *Alphitobius diaperinus*, *Locusta migratoria* and *Tenebrio molitor* for human consumption by the European Union has opened new opportunities to explore their secondary biological potential beyond protein content. This study proposes a comprehensive and comparative in silico analysis of bioactive peptides derived from these four insect species, targeting key enzymes involved in hypertension (the C-domain of somatic angiotensin-I converting enzyme, cACE), cardiac and pulmonary fibrosis (the N-domain of ACE, nACE), Alzheimer's disease (acetylcholinesterase, AChE, and butyrylcholinesterase, BChE) and type 2 diabetes (dipeptidyl peptidase 4, DPP4).
Reviewed protein sequences from each species were obtained from Swiss-Prot (UniProtKB) and submitted to the BIOPEP-UWM server for simulated gastrointestinal digestion, resulting in the generation of an extensive peptide library. Molecular docking was subsequently performed using GOLD software with the PLP score function against five therapeutic targets: nACE (PDB: 6EN5), cACE (PDB: 6F9T), AChE (PDB: 4EY7), BChE (PDB: 5NN0) and DPP4 (PDB: 4A5S). ADMET profiling was carried out via pepADMET to evaluate the resulting fragments intestinal absorption, blood-brain barrier permeability and toxicity. Candidates were selected based on target-specific criteria, prioritising sACE domain preference, DPP4 docking performance and predicted dual cholinesterase inhibition potential. ADMET-related descriptors were also integrated into the ranking to validate the biological relevance.
The computational screening process identified promising candidate peptides for each species. For A. domesticus, the peptides were DQQAEVN, IATTIF and AVQPCF for nACE; TGAQSL and EEPY for cACE; CPW as a dual ChE candidate peptide; and APEAK, TIGH and EQDK for DPP4. For A. diaperinus, the final candidates were IVTVTDGK, TVTAATGK and EEVVTL for nACE; PVPIDPW, DEQF and SSVIDCL for cACE; APK, VTIH and GESDL as dual ChE peptides; and AEGER, EVDR and ETTTL for DPP4. For L. migratoria, the selected peptides were DGETPIY, GDQQVQTL and AAPAVAK for nACE; EAPF, QGY and SQL for cACE; VSR, SQL and CGM as dual ChE candidate peptides; and ISESGK, TADPVN and QADDH for DPP4. For *T. molitor*, the selected peptides were GIEIGR, QTIAK and VDVVK for nACE; DDEESEK, VASPL and QIAF for cACE; VSR as a dual ChE peptide; and QPVSY, TPTDF and QTGEM for DPP4.
Collectively, these findings contribute to the characterisation of insect-derived bioactive peptides with potential relevance for cardiometabolic, fibrotic and neurodegenerative disease-related pathways. By identifying novel sequences across EU-authorised species, this study supports the development of functional foods that align with sustainable dietary transitions and the wider goals of the European Green Deal.

Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through UID/50006/2025, and from European Union, FEDER, through the projects EMPOWER (COMPETE2030-FEDER-00861800).
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Purification and mode of action of a new bacteriocin with activity against *Listeria monocytogenes* produced by *Enterococcus faecalis* L3A21K6

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Enterococcus faecalis L3A21K6 was previously isolated from an artisanal Azorean cheese (Pico cheese) and was found to produce an unidentified bacteriocin active against Listeria monocytogenes[1]. A bacteriocin operon coding for a novel class II lasso peptide, with 44% similarity to Propeptin 22, was revealed by whole genome sequencing[2].

The aim of this work was the purification of this bacteriocin and the investigation of its mode of action. An overnight culture of *E. faecalis* L3A21K6 was inoculated (1% v/v) into MRS broth and incubated at 30 °C for up to 72 h. Cell growth was monitored by measuring the absorbance at 540 nm. For antimicrobial activity assays, each sample was centrifuged (4500 × g, 10 min, 4 °C) and the resulting cell-free supernatant (CFS) was neutralized (pH = 6.5), filtered (0.22 µm) and subsequently tested against *L. monocytogenes* ATCC 7466 by the well-diffusion assay (WDA). The mode of action was assessed by conductivity assay, using *L. innocua* ATCC 33090 and the CFS, at 37 °C. For purification, the strain was inoculated (1% v/v) into MRS broth and incubated at 30 °C for 24h. The cells were harvested (4500 × g, 10 min, 4 °C) and the resulting CFS was neutralized (pH = 6.5) and filtered (0,22 µm). The bacteriocin was concentrated with 80% ammonium sulphate at 4 °C overnight, followed by centrifugation (4500 × g, 30 min, 4 °C). The resulting pellet was resuspended in phosphate-buffered saline (PBS), followed by dialysis (1 kDa, Spectra/Por6 dialysis membrane, Spectrum, USA) for 24 h at 4 °C. The dialysate was subjected to solid phase extraction (SPE) using two different cartridges: a C18 (Bond Elut – C18, VARIAN, Inc.) and a strong Cation Exchange (SCX LiChrolut, Merck). For the C18 cartridge, the dialysis fraction was loaded (flow-through), followed by 3 mL of 70% ethanol and 3 mL of 30% isopropanol. For the SCX cartridge, the dialysis fraction was loaded (flow-through), followed by 3 mL of wash buffer (NaH2PO4 20 mM, NaCl 0,2M, pH = 5.8) and 3 mL of elution buffer (NaH_2PO_4 20 mM, NaCl 1 M, pH = 5.8). Antimicrobial activity was assayed by WDA in all fractions and protein was quantified by the Lowry method.

Bacteriocin production increased during the growth phase, reaching 1067 AU/mL after 10 h, and this activity was sustained for up to 48 h. A 21% increase in conductivity was observed for bacteriocin-treated cells after 0.5 h, suggesting pore formation in the Listeria membrane. During purification, the highest specific activity was achieved with the cationic (SCX) cartridge, reaching 13700 AU/mg, which corresponded to a purification factor of 60. Further purification will be performed by reverse-phase liquid chromatography (RP-HPLC). Following complete purification, the peptide will be sequenced for structural confirmation, and its antimicrobial efficacy will be evaluated in relevant food model systems.

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Assessment of antimicrobial potential of propolis extracts for the artisanal cheese preservation

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Artisanal cheese production is a practice that has been passed for thousands of years, valued for its gastronomic qualities, sensory characteristics, and diversity. However, cheese has a complex microbiota that creates a high risk of spoilage and pathogen contamination. For this reason, there has been growing interest in strategies to extend the shelf life of these products, particularly antimicrobial molecules, edible films, and coatings.
Propolis, a chemically complex substance produced by bees, has high antimicrobial potential and, due to these properties, it has shown great potential as a natural preservative for cheese. Therefore, this work aimed to evaluate the antimicrobial activity of different propolis extracts against microorganisms previously isolated from cheese.
Seven microorganisms — Staphylococcus aureus, Escherichia coli, a mixed lactic acid bacteria culture, a mixed yeast culture, Aspergillus sp., Cladosporium sp., and Galactomyces geotrichum — were isolated from four types of cheese (goat, sheep, a cow–sheep mixture, and a cow–sheep mixture with walnuts) to test the antimicrobial activity of the propolis extracts.
Raw propolis was collected in Bragança, Portugal, and ultrasound-assisted extraction was performed using different green solvents: 80% propylene glycol, 70% ethyl lactate, and a mixture containing 36.5% propylene glycol and 23.5% ethyl lactate. A 70% ethanol solution was used as the benchmark solvent. The resulting extracts were chemically characterized by determining their total phenolic and flavonoid contents and were evaluated for antimicrobial activity. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using spectrophotometric methods, whereas antibacterial and antifungal activities were assessed using the broth microdilution and agar dilution methods, respectively. The extracts obtained using green solvents exhibited extraction efficiencies comparable to those of the hydroethanolic benchmark extract, reaching TPC and TFC values of 167 mg GAE/g and 65 mg QE/g of raw propolis, respectively. Overall, the ethyl lactate extract showed the strongest antimicrobial activity, followed by the ethanolic extract. However, the ethanolic extract was more effective against bacteria than the ethyl lactate extract.
In addition to these antimicrobial activity results, the propolis extracts will be applied to or incorporated into artisanal cheese to evaluate how different microorganisms interact with them during the various stages of cheese ripening.

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Hyperspectral Imaging as a Non-Destructive Tool for Protein Prediction in Insect Flours

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The increasing demand for sustainable alternative protein sources has driven the development of insect-based ingredients for human nutrition due to their high nutritional value, functional properties, and lower environmental impact compared with conventional livestock production[1]. However, conventional methods used to determine protein content, such as the Kjeldahl method, are destructive, time-consuming, and require the use of chemical reagents. In this context, Hyperspectral Imaging (HSI) has emerged as a promising analytical technique for rapid, non-destructive, and reagent-free characterization of food matrices by combining spatial and spectral information.

Recent studies have demonstrated the feasibility of near-infrared hyperspectral imaging coupled with chemometric methods for predicting protein content in edible insects[2]. Building on these findings, this study evaluated the use of HSI for protein prediction in insect flour. The protein content of the flour was determined using the reference Kjeldahl method, yielding a value of 39.38%, confirming its high nutritional value. Hyperspectral images were acquired using the Specim FX10 (400–1000 nm) and Specim FX17 (900–1700 nm) cameras, providing complementary spectral information from the visible and near-infrared (VNIR) and short-wave near-infrared (SWIR) regions. Spectral data were pre-processed and analysed using chemometric techniques, including Principal Component Analysis (PCA) for exploratory data analysis and Partial Least Squares Regression (PLS-R) for quantitative model development.

Based on previous studies, protein-rich insect flours are expected to exhibit characteristic absorption features in the NIR and SWIR regions associated with N–H, C–H, and O–H bond overtones and combination bands. These spectral signatures are anticipated to support the development of accurate chemometric models for protein prediction, consistent with the findings reported the literature.2 Considering the large volume of hyperspectral data generated, dimensionality reduction strategies were also explored to improve computational efficiency while preserving relevant spectral information, following recent advances reported for large HSI datasets[3].

The analytical protein value obtained by the Kjeldahl method was used as the reference for the calibration and validation of the chemometric models. Model performance was assessed by comparing the predicted protein values with the analytical results. The combination of spectral information from the Specim FX10 and FX17 cameras is expected to improve the robustness of protein prediction compared with the use of individual sensors. This approach supports the application of hyperspectral imaging as a rapid, non-destructive, and environmentally friendly tool for protein determination and quality assessment of insect-based ingredients.

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Development and characterization of a faba bean-based extruded snack naturally sweetened with fruit

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Growing consumer demand for healthy, sustainable, and clean-label foods has driven interest in pulse-based extruded snacks as nutritious alternatives to conventional cereal products. Pulses offer high protein and fiber contents and a favorable sustainability profile, yet their incorporation at high levels poses technological challenges for expansion and texture. This work developed an extruded snack with high pulse content (70% faba bean starch), combined with rice flour, and naturally sweetened with fruit, using date combined with strawberry, raspberry or carob. Formulations were adjusted to a moisture level of 50% and processed using a single-screw cold extruder (Nudelmaschine PN 100, HAUSSLER, Deutschland). The extrudates were characterized through physicochemical (pH, °Brix, water activity), nutritional (proximate composition), and sensory analyses (flavor, texture and appearance, evaluated by 25 untrained panelists). Results show that nutritionally, the inclusion of 70% faba bean starch achieved a protein content of approximately 10% and fiber content of 4-8%. Moisture content (4-7%) and water activity (0.28-0.45) values suggest physicochemical stability. Sensory evaluation showed good overall acceptability with an average score of 4 on a 5-point scale. Fruit incorporation delivered sweetness and characteristic flavor notes without added refined sugar, supporting the clean-label objective. Hence, snacks with faba bean starch, combined with rice flour and fruit-based sweeteners, can yield a nutritionally favorable and sensorially acceptable extruded snack. These findings support the use and valorization of pulse-based ingredients in clean-label snack development and highlight fruit-based sweetening as a viable strategy for reducing refined sugar in extruded products.

Pomegranate Peel Extracts as Bioactive Ingredients for Food Preservation: Chemical Characterization and Antibacterial Activity

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Food waste represents a major global challenge, with fruit and vegetable by-products offering an attractive source of high-value bioactive compounds. Among these, pomegranate peel is particularly rich in phenolic constituents with promising applications in natural food preservation[1]. In this study, freeze-dried pomegranate peel was extracted with water and hydroalcoholic solvents (50:50, v/v), and the resulting extracts were characterized regarding their phytochemical composition, antioxidant capacity, stability, and antibacterial activity.

Hydroalcoholic extracts exhibited the highest total phenolic (up to 236.0 mg GAE/g DW for methanol and 205.7 ± 8.5 mg GAE/g DW for ethanol) and flavonoid contents (81.3 ± 1.7 and 77.1 ± 9.1 mg CE/g DW, respectively), consistent with their greater ferric reducing antioxidant power (FRAP). In contrast, all extracts displayed comparable DPPH radical scavenging activity, with IC_{50} values ranging from 5.17 to 5.94 µg/mL. HPLC analysis confirmed the presence of phenolic compounds, although at lower concentrations than those estimated by colorimetric assays. LC–MS analysis identified α- and β-punicalagin as the predominant compounds, together with other punicalagin derivatives, ellagic acid and its glycosylated derivatives, while HHDP-D-glucose was detected exclusively in hydroalcoholic extracts. Extract stability under different temperature, light, and pH conditions was also assessed to evaluate their suitability for food-related applications.

Antibacterial activity was evaluated against Staphylococcus aureus ATCC 29213. All extracts exhibited pronounced antibacterial activity, with the aqueous extract demonstrating efficacy comparable to that of the ethanolic and methanolic extracts. At concentrations of 4 and 8 g/L, bacterial counts were reduced by approximately 4 and 6 $\log_{10}$ CFU/mL, respectively. These findings highlight pomegranate peel as a sustainable source of bioactive compounds for natural food preservation and demonstrate that water is an effective green extraction solvent, supporting the development of environmentally friendly technologies for food applications.

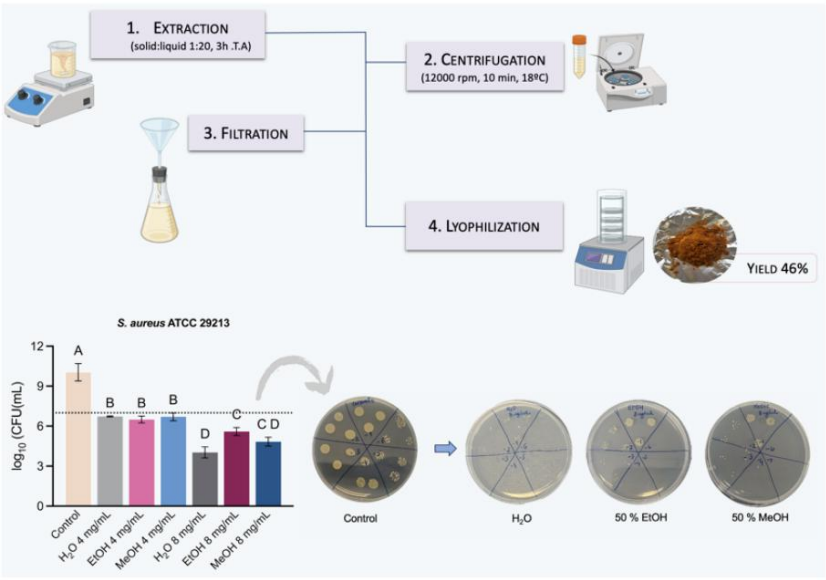


Figure 1. Extraction of pomegranate peel and antibacterial efficacy of the resulting extracts against Staphylococcus aureus ATCC 29213. The dotted line marks the 3-$\log_{10}$ reduction threshold (99.9% reduction in viable bacterial cells).

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Development of a Single LC-MS/MS-SRM Method for the Determination of Water- and Fat-Soluble Vitamins in Fortified Food Matrices

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Vitamins are essential micronutrients involved in numerous metabolic pathways and physiological processes and therefore play a crucial role in maintaining human health[1]. These bioactive compounds are classified into two groups according to their solubility: water-soluble vitamins (B-complex vitamins and vitamin C) and fat-soluble vitamins (A, D, E, and K) (Figure 1). Their markedly different physicochemical properties, together with their low concentrations and susceptibility to degradation, make their simultaneous determination in food matrices analytically challenging[2]. Consequently, most analytical methodologies are designed for either water- or fat-soluble vitamins, requiring different chromatographic conditions.

This work aimed to develop and optimize a single liquid chromatography–tandem mass spectrometry (LC-MS/MS) method operating in Selected Reaction Monitoring (SRM) mode for the determination of water-soluble vitamins (B1, B2, B3, and B9) and fat-soluble vitamin (vitamin D3) in fortified fruit matrices. Owing to the distinct physicochemical characteristics of the target analytes, separate sample preparation procedures were employed. Water-soluble vitamins were extracted using water as the extraction solvent, whereas vitamin D3 was recovered through a methanol: dichloromethane (1:1 (v/v) solvent mixture[3]. After ultrasound-assisted extraction (15 minutes) and centrifugation (13,400 rpm, 15 minutes, 4 °C), all extracts were analyzed using the same optimized chromatographic method on an Acclaim™ RSLC 120 C18 column coupled to a Thermo Scientific TSQ Altis Plus triple quadrupole mass spectrometer operating in SRM mode. Chromatographic separation, mobile-phase composition (solvent A - 0.1% formic acid in water (v/v) and solvent B - 0.1% formic acid in methanol (v/v)), gradient elution, and mass spectrometric parameters were systematically optimized to maximize sensitivity and selectivity across all analytes. Calibration curves for all analytes exhibited excellent linearity (R^2 > 0.9959) over the evaluated concentration ranges. Limits of detection (LOD) and quantification (LOQ) were also determined for all target vitamins.

The developed LC-MS/MS-SRM platform demonstrates that a single chromatographic method can be successfully applied to the quantitative determination of both water- and fat-soluble vitamins following class-specific sample preparation, providing a versatile analytical tool for the characterization of fortified food products.

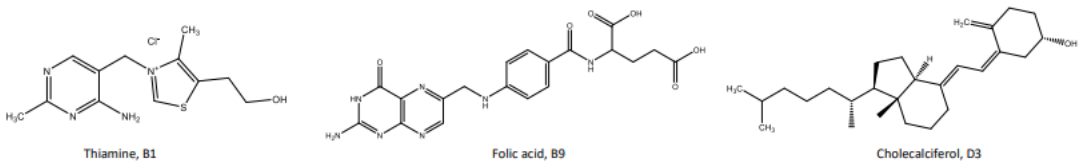


Figure 1 – Representative chemical structures of water-soluble (vitamins B1 and B9) and fat-soluble (vitamin D3) vitamins, highlighting their structural diversity and distinct physicochemical properties.

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Modulating Photodynamic Behavior of Food Chromophores with Blue Light: A Comparative Study of Curcumin, Riboflavin and Quercetin

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Many food-derived bioactive compounds are traditionally valued for their antioxidant and protective properties. However, several naturally occurring chromophores can exhibit distinct biological behavior upon light exposure. Curcumin, riboflavin and quercetin have been extensively investigated for their antioxidant, photoprotective and other beneficial properties, while their ability to absorb visible or ultraviolet light and generate reactive oxygen species has also led to their investigation as photoactive compounds and photosensitizers[1-3]. This apparent antioxidant/pro-oxidant duality is particularly relevant for compounds capable of absorbing blue light, yet direct comparisons of their photodynamic performance remain challenging because of substantial differences in experimental conditions across studies. Here, curcumin, riboflavin and quercetin were comparatively evaluated under standardized blue-light irradiation to investigate how their photochemical properties translate into cellular photodynamic effects.

The three chromophores were characterized in terms of UV–visible absorption profiles and molar extinction coefficients, followed by evaluation of photostability by UHPLC after irradiation. Intracellular singlet oxygen-related response was assessed by confocal imaging using SO Green™ 520AM Singlet Oxygen Sensor. Photodynamic activity was evaluated in B16-F10 melanoma cells using a resazurin-based viability, with two treatment configurations: removal of extracellular chromophore after a 1 h pre-incubation or direct irradiation (405 nm, 10 min) in the presence of the compound. Longer-term effects on cellular proliferative capacity were further assessed by clonogenic analysis.

Under blue light, curcumin displayed the strongest photodynamic activity causing approximately 70% loss of B16-F10 viability at only 3.13 μM, whereas riboflavin and quercetin produced negligible effects. In contrast, when the compounds were retained during irradiation, both riboflavin and quercetin exhibited pronounced concentration-dependent phototoxicity, reaching more than 85–90% loss of viability at 12.5–25 μM. Curcumin-induced phototoxicity was further supported by its strong effect on long-term proliferative capacity: 1.5 μM curcumin reduced colony number by approximately 76% relative to blue-light-irradiated cells in the absence of compound.

The photochemical and cellular responses revealed distinct behavior among the three chromophores. Curcumin underwent partial depletion of the parent chromatographic peak upon irradiation, while quercetin showed comparatively greater stability with no apparent formation of additional chromatographic species. Riboflavin exhibited the greatest photochemical instability, with substantial depletion of the parent peak accompanied by the formation of new species, namely lumichrome and lumiflavin.

Interestingly, intracellular SO Green fluorescence was markedly higher upon riboflavin irradiation, suggesting riboflavin as the best singlet oxygen producer, followed by quercetin, whereas curcumin produced a comparatively weak signal. Thus, the magnitude of intracellular singlet oxygen-related fluorescence did not directly predict phototoxicity. The pronounced phototoxicity of curcumin following removal of the extracellular compound may reflect more efficient cellular retention and/or localization at photodamage-sensitive sites, potentially facilitated by its lipophilic character. Moreover, curcumin is capable of generating reactive oxygen species beyond singlet oxygen, suggesting that its pronounced phototoxic response may result from the combined contribution of multiple ROS rather than singlet oxygen alone.

Overall, curcumin exhibited the most effective and persistent photodynamic response, combining strong acute phototoxicity with pronounced inhibition of long-term colony formation. Riboflavin showed the highest intracellular singlet oxygen-related response but substantially lower phototoxicity when extracellular compound was removed. By applying identical irradiation conditions across structurally distinct food-associated chromophores, this study provides a more meaningful basis for comparing their relative photodynamic performance in blue-light-mediated photodynamic approaches, particularly for skin-related applications and light-based antimicrobial strategies.

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Scale-up of an enzyme-assisted process for integrated protein and lipid recovery from wheat germ

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Cereals, particularly wheat, are a fundamental part of the global diet, but its processing generates significant co-products, namely wheat germ (WG), yielding, annually and globally, approximately 25 million tons[1]. This co-product remains largely underutilized. Wheat germ contains proteins, lipids, and bioactive compounds, making both by-products promising for sustainable valorization[2]. Their applications range from functional foods and high-protein pasta to oil-in-water cosmetic formulations and biodegradable packaging materials[3].So, the wheat co-product can be divided into two categories: wheat germ oil (WGO) and wheat germ proteins (WGP).
However, developing a process that handles both fractions while retaining their compositional value requires careful selection and subsequent evaluation under larger processing volumes[3]. Recovering both fractions through a single process could improve its valorization, provided that the treatment remains effective when transferred beyond laboratory scale. This study evaluated the scale-up of an enzyme-assisted treatment previously selected through comparative screening.
Wheat germ containing 25.9% protein was processed in a 5-L reactor at a germ-to-buffer ratio of 1:5. The selected treatment combined 1% Alcalase, 2% Tergitol 15-S-7 and 2% vitamin E and was conducted at 50 °C for 4 h. It was compared with untreated wheat germ and wheat germ treated with Alcalase alone. Process performance was assessed through mass recovery, protein extraction yield and fatty-acid concentration in the recovered material.
The combined treatment achieved the highest mass recovery (49.9%) and protein extraction yield (79.2%) among the three conditions. The recovered fraction had a measured fatty-acid concentration of 197.2 mg/mL, and approximately half of the detected fatty acids were unsaturated (100.2 mg/mL).
These findings indicate that the treatment selected during laboratory screening maintained its capacity to recover protein and lipid components at reactor scale. Further work should establish the composition and intended applications of the recovered fractions. Furthermore, the insights gained from this study could serve as a foundation for future research on bioavailability, consumer acceptance, and regulatory feasibility, further strengthening the potential impact of wheat-derived bioactive compounds.

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Antioxidant enzymes as slaughter-stress biomarkers in Tenebrio molitor larvae: implications for powder quality and safety

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Insect slaughter methods are rarely evaluated for their effects on animal welfare, safety, and product quality. In this study, six slaughter treatments were compared in T. molitor larvae: freezing (-24 °C), blast-chilling (-45 °C), and blanching (100 °C), each under two conditions. After slaughter, a portion of the larvae was stored at -80 °C to determine the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The remaining larvae were dried at 80 °C and ground. The resulting powder was evaluated for microbial load over six months of storage, oxidative stability of oil, colour, centesimal composition, and techno-functional properties. The slaughter method significantly influenced the profile of antioxidant enzymes and the initial microbial load. Rapid blast-chilling resulted in the lowest bacterial counts and a favourable stress biomarker profile. Lipid oxidation was associated with standard post-slaughter thermal processing (bleaching and oven drying) rather than the slaughter stage itself and was lower after freezing. These results suggest that slaughter and drying should be optimised together. Rapid blast-chilling provided the most balanced outcome for welfare and safety, though not for lipid oxidation. This study reports the first use of the SOD/CAT/GPx panel as a slaughter-stress biomarker in an edible insect.

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Consumer conceptualisation and acceptance of laboratory-grown meat: qualitative insights from Portugal

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Emerging food technologies raise questions about how consumers understand and evaluate new foods, as well as scientific questions concerning their production, composition and quality. Research on consumer acceptance of laboratory-grown meat highlights the importance of perceived benefits and risks[1] as well as perceived naturalness and trust[2]. The terminology used to describe the product can also influence consumer perceptions[3]. This study explored how Portuguese consumers conceptualise laboratory-grown meat and the factors associated with acceptance or reluctance to consume it. The research followed an exploratory sequential mixed-methods design, in which the qualitative interview phase informed the development of a questionnaire for the subsequent quantitative phase, which is currently underway.

The qualitative findings presented here are based on individual semi-structured interviews with 20 Portuguese adult participants aged 22 to 78 years, recruited through convenience sampling. The interviews were conducted between June and July 2025 and explored familiarity with laboratory-grown meat, perceived risks and benefits, willingness to consume, preferences regarding product terminology, and information needs. As a complementary activity, participants were shown cards with images of minced meat labelled “organic production”, “intensive production”, “laboratory-grown meat”, “3D meat”, “artificial meat”, and “cultivated meat” and were asked to rank the cards separately according to three criteria: perceived naturalness, purchase intention, and anticipated taste. The interviews were recorded, transcribed, and subjected to thematic analysis that combined deductive and inductive coding, with support from NVivo 15.

The analysis identified diverse perspectives, including expressions of curiosity and openness, as well as uncertainty and rejection. Favourable accounts highlighted interest in food innovation and perceived potential benefits for animal welfare and environmental sustainability. However, recognition of these potential benefits did not necessarily translate into an unreserved willingness to consume the product. Participants identified safety, expected sensory attributes, nutritional quality and price as relevant considerations in deciding whether to consume it. Naturalness also featured in the associations elicited by different product names. “Cultivated meat” stood out among the preferred designations, being associated with greater neutrality and, among the technological alternatives evaluated, with the highest perceived naturalness, whereas references to laboratory production and synthetic characteristics prompted reservations in some accounts. Regarding information needs, participants highlighted the importance of understanding the product’s production process, composition, origin and potential risks and benefits. Institutional trust and access to credible information were also mentioned in relation to perceived safety and willingness to try it.

These findings suggest that the conceptualisation and acceptance of laboratory-grown meat combine assessments of perceived benefits and risks with meanings attributed to the product and its technological origin. Openness to innovation can coexist with reservations about naturalness, safety and expected food characteristics. The findings highlight the relevance of terminology and trust in understanding these perspectives. Within the field of food science, these findings may help align the development of emerging foods more closely with consumers’ perceptions, concerns, and information needs. The study provides a contextual understanding of the perspectives expressed by this sample, without estimating the prevalence of attitudes in the Portuguese population.

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XVIII Encontro de Química dos Alimentos

Coimbra | 14 - 16 de outubro'26

EMBALAGEM: DO BARRO AO BIOPLÁSTICO INTELIGENTE



Investigadora Sénior da Aragonese Foundation for Research and Development (ARAID), integrando o Analytical Research Group (GUIA) do Aragon Institute of Engineering Research (I3A), da Universidade de Saragoça, em Espanha. A sua atividade científica centra-se na microbiologia aplicada, nanotecnologia e química analítica, com particular enfoque no desenvolvimento de soluções inovadoras para a indústria alimentar. Ao longo dos últimos 15 anos, tem participado e liderado diversos projetos dedicados ao desenvolvimento de embalagens ativas, nomeadamente soluções com propriedades antimicrobianas, antioxidantes e de remoção de etileno. A sua investigação abrange a encapsulação de agentes ativos, o desenvolvimento de materiais biodegradáveis ou recicláveis e a avaliação in vitro e de prova de conceito destas soluções, incluindo a análise da sua segurança química.

Active Packaging strategies for food quality and safety

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The Analytical Research Group (GUIA) at the University of Zaragoza is a benchmark in the development of active packaging and advanced food preservation technologies. During this lecture, we will present our most recent scientific contributions encompassing the integration of bioactive natural compounds, the valorisation of industrial by-products, and the rigorous chemical safety evaluation of emerging food-contact materials.

One of our focus areas is replacing conventional plastics with biodegradable matrices, such as polycaprolactone (PCL), polyhydroxyalkanoates (PHA/PHB), or synthetic and natural biopolymer coatings that we have successfully used for the design of active materials capable of delivering sustained release of antimicrobial agents. Some of our recent work includes biodegradable blends containing ε-polylysine[1] to control critical pathogens such as Listeria monocytogenes in highly perishable products (like fresh salmon), successfully inhibiting bacterial growth, and PHB films containing cylodextrin inclusion complexes with benzyl isothiocyanate (BITC) to control Campylobacter spp. growth[2].

Additionally, we also explore essential oils (such as thyme, oregano, or cinnamon leaf) and volatile organic compounds to act via the vapor phase. This approach prevents direct, massive contact between the additive and the food surface, minimizing taste and aroma alterations. Recently, we have formulated emulsions to be deposited onto paper substrates using cationic starches. These active systems demonstrated high antifungal efficacy against Botrytis cinerea, substantially extending the shelf life of fresh produces such as tomatoes and reducing waste in the supply chain. Since volatile compounds can be very unstable (premature volatilization, light sensitivity, or thermal degradation), we also develop cyclodextrin nanostructures (nanosponges) that serve as controlled-release reservoirs for antimicrobials and antioxidants, while also being explored as active ethylene scavengers[3], thus delaying fruit and vegetable ripening.

In line with circular economy principles, some of our recent work addresses the incorporation of recycled materials—such as cellulose nanofibers derived from industrial pulp to be used as active coatings for cellulose films. To provide a more complete view on how the active food packaging materials behave, we also evaluate their chemical risk assessment to ensure that the materials developed can be safely used as food contact materials according to European legislation 10/2011.

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Smart packaging with printed biobased colorimetric indicator for real-time assessment of fish freshness

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Approximately 35% of fish products are wasted at the retail and consumer stages, largely because fixed expiration dates do not accurately reflect the actual freshness of the product, resulting in the disposal of fish that may still be suitable for consumption[1]. Smart packaging technologies, such as colorimetric indicators, offer a promising alternative by enabling real-time monitoring of food quality. In particular, pH-responsive indicators can detect volatile amines generated during fish spoilage and provide a visual indication of freshness through a color change.

In this work, a cost-effective colorimetric indicator was developed via screen-printing directly onto plastic packaging for the monitoring of salmon freshness. The sensing ink was formulated using anthocyanins extracted from red cabbage as natural pH-sensitive pigments and incorporated into a bio-based matrix composed of starch and water-soluble chitosan. The rheological properties of the ink were optimized by adjusting the polysaccharide composition to ensure suitable viscosity for printing and the production of homogeneous, highly saturated indicator patterns. The indicators were susceptible to fading under external environmental conditions, but this issue was mitigated by storing them in humidity-free and N_2-rich environments. To improve stability under the high-humidity conditions typically encountered in fish packaging, a thin polydimethylsiloxane (PDMS) coating was applied to the printed indicators. This protective layer effectively prevented wash-off while maintaining permeability to volatile amines.

The developed indicators exhibited a clear and reproducible color response when exposed to different concentrations of trimethylamine and to salmon undergoing spoilage, demonstrating their ability to track freshness changes. The indicators showed a visible color change even at low concentrations (50 mg/L), well below the maximum TMA threshold (109 mg/L). These results highlight the potential of the proposed indicator as a sustainable and reliable alternative to conventional expiration dates. By enabling real-time freshness monitoring, this bio-based sensing platform could support more informed decision-making by consumers and retailers, contributing to improved food safety and a reduction in food waste. Furthermore, the use of screen-printing provides a scalable and economically viable manufacturing route, facilitating the integration of such indicators into commercial food-packaging systems.

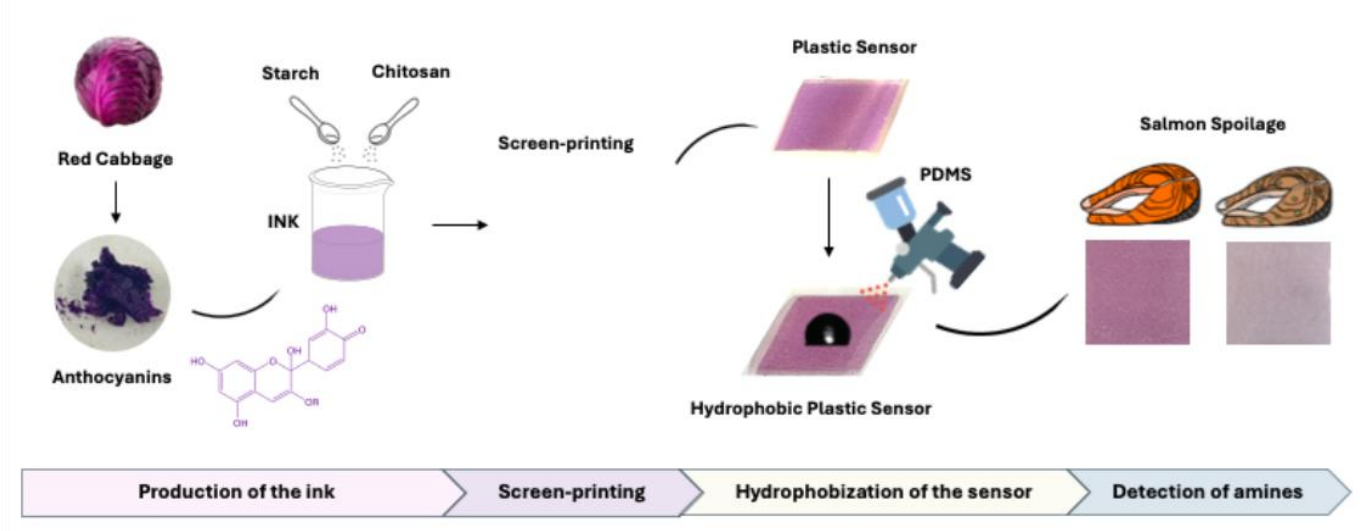


Figure 1 – Schematic representation of the biobased pH-responsive indicators production for fish freshness monitoring.

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The Clean Label Challenge in Ripened Cheese: Whey Protein-Based Active Coatings with Grape Skin Extracts for Natural Preservation

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The growing demand for Clean Label foods is driving the search for natural alternatives to synthetic preservatives. In ripened cheeses, natamycin is the most widely used surface antifungal. Simultaneously, the valorisation of agro-industrial by-products, such as whey from cheese making and grape pomace from winemaking, remains a key challenge for the circular economy[1]. This study aimed to develop an active edible coating based on whey protein, incorporated with grape skin extracts from red and white varieties, to inhibit surface microbial growth on ripened cheese and ideally eliminate the need for synthetic biocides.

Grape pomace from both red and white varieties was fractionated to isolate the skins. A hydroalcoholic extraction of bioactive compounds was optimised by Response Surface Methodology (RSM) using a Box-Behnken design, with ethanol concentration (20–80 %v/v), temperature (30–60 °C) and extraction time (30–120 min) as independent variables. The film-forming solution was prepared from a nanofiltered and freeze-dried whey protein fraction (95 % protein content), using glycerol as plasticiser, and incorporating 1 % (v/v) of the optimised ethanolic extract (55 % ethanol at 60 °C, with extraction times optimised for each variety). Films were obtained by casting and characterised in terms of water vapour transmission rate, solubility, water absorption, gloss, transparency, opacity, colour difference (ΔE), elastic modulus, antioxidant capacity (DPPH and FRAP assays), FTIR spectroscopy, and surface morphology by SEM. Antimicrobial activity was evaluated by the disk diffusion method against Penicillium commune, Aspergillus niger and Pseudomonas fluorescens.

The films displayed water vapour transmission (950 g·m2·day-1) and mechanical properties compatible with the cheese ripening process, allowing adequate gas exchange. The incorporation of grape skin extracts conferred antioxidant capacity, as confirmed by DPPH (red grapes 87.7 and white rapes 88.3 µmol TE.g-1) and FRAP (red grapes 77.1 and white 57.8 µmol TE·g-1). FTIR spectra suggested molecular interactions between the protein matrix and polyphenols, while SEM images revealed homogeneous, crack-free surfaces. In the disk diffusion assays, inhibition zones against both moulds were comparable to those obtained with natamycin under the same experimental conditions. Importantly, the active films also inhibited P. fluorescens, a spoilage bacterium not sensitive to the commercial biocide.

As such, a pilot-scale trial was conducted on Serpa PDO cheese (after 15 days of ripening, which corresponds to half the time) by spraying the film-forming solution on the cheese surface. Surface microbial development, physicochemical parameters (moisture, water activity, pH, colour, texture), and sensory attributes (flavour, aroma, colour) were monitored over the ripening period and compared with both untreated controls and natamycin-treated samples. In this trial, no significant differences in physicochemical parameters were observed between coated and control cheeses. Sensory analysis did not reveal any perceptible alterations in flavour, aroma or colour, a result corroborated by ΔE values below 2, which are typically considered not perceptible to the human eye.

These preliminary findings demonstrate the technical feasibility of whey-protein-based active coatings with antifungal efficacy similar to natamycin, without compromising the sensory quality of ripened cheese. The approach simultaneously valorises two major agro-industrial by-products, whey and grape pomace, contributing to a more sustainable and circular food production system.

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Active chitosan films enriched with Opuntia ficus-indica extracts: does it contribute to poultry meat preservation?

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Food safety and the reduction of food waste are major challenges in the food industry, particularly for highly perishable products like poultry meat. Simultaneously, the environmental impact of fossil-based plastics has intensified the demand for sustainable packaging alternatives. Biopolymer-based active packaging, such as chitosan films, offers a sustainable alternative to synthetic plastics while providing intrinsic antimicrobial properties.[1] Incorporating natural agricultural by-products, such as the betalain-rich extract of *Opunti ficus-indica* fruits, can further enhance these protective effects and promote a circular bioeconomy. This strategy valorizes agro-industrial waste to promote a circular bioeconomy while creating a synergistic active barrier against microbial spoilage.

In this context, chitosan-based films incorporated with hydroalcoholic extracts of prickly pear peel (*Opuntia ficus-indica*) at two concentrations (1% and 2%) were developed, and their efficacy in preserving fresh chicken meat during 14 days of refrigerated storage was evaluated. The extracts were previously characterized regarding their phenolic compound profile, betalain content and antioxidant activity. Meat quality was monitored through microbiological parameters (mesophilic, psychrotrophic and *Enterobacteriaceae* counts) and physicochemical parameters, including pH, titratable acidity, moisture, color, total volatile basic nitrogen and lipid oxidation (TBARS).

Chitosan films demonstrated significant antimicrobial efficacy compared to the control (unwrapped meat). At the end of storage time, mesophilic counts decreased by 2.7 log CFU/g, and psychrotrophic counts by 2.8 log CFU/g compared to the control. For *Enterobacteriaceae*, a reduction from 6.55 log CFU/g in the control to approximately 4.94 log CFU/g in samples coated with chitosan and to 3,95 log UFC/g in the samples containing 2% extract was observed, demonstrating that extract incorporation improved film activity. The incorporation of extracts also showed a positive effect on pH stabilization, maintaining lower values throughout storage, whereas the pH of the control increased from 5.69 to 6.65. Regarding total volatile basic nitrogen, the control reached approximately 0.05 g N/100 g meat at the end of storage time, while samples coated only with chitosan showed lower values (≈0.04 g N/100 g meat). However, in terms of TBARS, films containing extracts, particularly at 2%, presented higher values, reaching approximately 0.40 mg MDA/kg meat, higher than what was observed for control (0.19 mg MDA/kg meat). The significant increase in TBARS values with extract incorporation suggests a possible pro-oxidant effect under the conditions studied. Yet the results are low, which do not compromise the use of this poultry meat after cooking. Overall, the developed films demonstrate potential as a sustainable solution for preserving fresh chicken meat.

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Biofilms containing cutin oligomers for applications in food packaging

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The growing environmental concerns associated with the extensive use of petroleum-based plastics have intensified the search for sustainable and eco-friendly alternatives. Conventional plastics are highly resistant to degradation and contribute significantly to environmental pollution across terrestrial, freshwater and marine ecosystems, where they progressively fragment into micro- and nanoplastics that persist, accumulate, and pose risks to ecosystems and human health.[1] As a result, bioplastics derived from renewable resources have emerged as a promising strategy to reduce the environmental impact of plastic waste and support the transition toward a circular bioeconomy. Natural biopolymers, such as polysaccharides and plant-derived polyesters, represent attractive raw materials for producing biodegradable and bio-based plastics[2], as they are renewable, widely available, and often sourced from agricultural by-products and industrial residues, contributing to resource valorization and waste reduction. Cutin, for example, is a plant-derived biopolyester found in abundance in tomato peel. The highly hydrophobic nature of this biopolymer gives it strong potential for use in bio-based materials, where water-repellent properties are desired, making it particularly relevant for improving the storage and preservation of perishable foods.

In this work, cutin oligomers (COMs) extracted from tomato pomace were incorporated into film-forming solutions to produce bio-based materials intended for food packaging applications. High-methoxyl pectin, carboxymethyl cellulose, and glycerol were used to prepare the film-forming matrix, with variable conditions and proportions tested. COM solutions were then dispersed in corn oil. Oil-in-water (O/W) emulsions were prepared by homogenizing the oil phase, containing the COMs with water 1:9, v/v) in the presence or absence of a food-grade stabilizer (e.g., soy lecithin), under different mixing conditions. The ensuing emulsions were subsequently mixed with the film-forming matrix at different concentrations. The materials were dried under different temperatures.

Biofilms containing COMs exhibited good transparency and a slightly yellowish appearance (Figure 1). The incorporation of COMs delayed the dissolution of the biofilms in water, due to the highly hydrophobic nature of cutin oligomers. This increased water resistance highlights their potential for the development of sustainable food packaging materials with enhanced water-repellent properties. Characterization of the bio-based materials (e.g. microscopy and spectroscopy) is ongoing to establish fundamental correlations between composition and material properties, prioritizing biodegradability and surface and thermal properties.

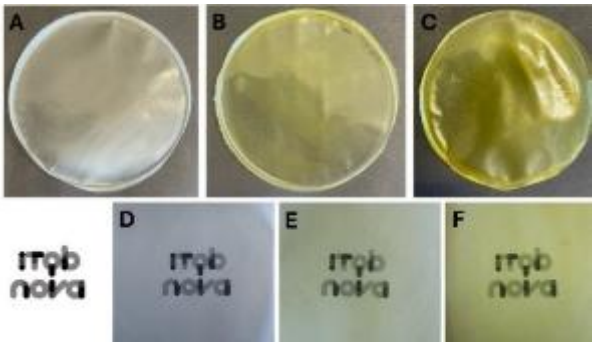


Figure 1. Physical appearance and transparency of films produced from HMP/CMC/glycerol blends and emulsions loaded with cutin oligomers (COMs). (A-D) Control: films without COMs. (B-E) Films containing 1.5% (w/w) COMs. (C-F) Films containing 3.0% (w/w) COMs.

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From food industry byproducts to water tolerant starch-based bioplastics: a mechanochemistry approach

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Food industry generates several secondary raw materials that have been increasingly recognised as valuable feedstocks for the development of biobased materials. Among these, starch-rich potato washing slurries have been explored as renewable sources for starch-based films production due to starch film-forming capacity and thermoplastic behaviour. However, these materials remain highly hydrophilic and brittle, which limits their suitability for food packaging applications. To overcome these drawbacks, research has focused on strategies to enhance flexibility and water resistance, including the incorporation of triacylglycerides derived from spent frying oil (SFO), which can participate in alkaline-assisted transesterification reactions.[1] Building on these advances, this study investigates a mechanochemistry-assisted approach to promote transesterification between starch hydroxyl groups and the fatty acyl chains present in SFO, using potassium hydroxide (KOH) as catalyst. By leveraging two abundant food-industry side streams, the aim was to produce water-tolerant starch ester bioplastics with enhanced mechanical performance. High-purity commercial potato starch was used as reference. Both starches were processed in native dry from and as pregelatinized starch, the latter prepared to enhance granule disruption and hydroxyl groups accessibility for chemical modification. Their similar amylose content (ca. 20%) provided a consistent basis for evaluating the impact of SFO/KOH modification process. Films produced through mechanochemical modification with SFO/KOH exhibited markedly improved performance relative to neat starch-based films (both native and pregelatinized recovered and commercial starches), including higher stretchability and elasticity (both 1–2×), and hydrophobicity (> 90°). Notably, films derived from recovered pregelatinized starch reached water contact angles around 120°, demonstrating that food-industry byproducts can yield bioplastics with superior water tolerance, surpassing commercial potato starch. ATR-FTIR analysis confirmed the incorporation of fatty acyl chains through the carbonyl band at 1740 cm^{-1}, suggesting starch-ester formation. Overall, this work highlights a promising green pathway for the food sector, showing that industrial byproducts can be transformed into functional, starch-based films with enhanced hydrophobicity. By integrating waste valorisation with bioplastic development, this approach reinforces the role of the food industry as a driver of circularity and sustainable material innovation.

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Active Whey Protein-Based Films Incorporated with Chestnut Pellicle Extract: Physicochemical Characterization and Antioxidant Effectiveness

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The accumulation of petroleum-based plastics has raised increasing concerns, due to their limited biodegradability and potential migration of harmful additives. In this context, the development of active biodegradable food packaging has gained increasing attention, allowing the incorporation of bio-based materials to create biodegradable films capable of delaying food deterioration and extending their shelf life. [1,2] This work aimed to develop antioxidant whey protein-based films, through the incorporation of chestnut pellicle, i.e. chestnut inner skin (CIS), a valuable yet underexploited by-product of the nut processing industry.

The whey protein-based films were prepared using the casting method, with the incorporation of 0% (control), 1% (w/w) and 2% (w/w) of the CIS extract. CIS antioxidant compounds were extracted through solid-liquid extraction with the Soxhlet's apparatus, and it was selected for film incorporation due to its high phenolic content and great antioxidant activity, as determined in a previous study. The efficacy of the obtained films in preventing lipid oxidation was evaluated through the Thiobarbituric Acid Reactive Substances (TBARS) assay, using raw salmon as the food model. The antioxidant capacity of the films and the migration of antioxidant compounds were analyzed using a food simulant (95% ethanol). The antioxidant properties were determined through the Folin-Ciocalteau, aluminum chloride, DPPH radical scavenging activity and β-carotene bleaching inhibition methods.[3]

The films were also characterized regarding their physicochemical and barrier properties, including thickness, transparency and opacity, moisture content, solubility, swelling capacity, and water vapor permeability (WVP).

TBARS results confirmed a delay in lipid oxidation compared to control samples, with 1% extract films showing the best results (0.20 mg MDA Eq./ kg sample, at day 4) compared to the control films (0.45 mg MDA Eq./ kg sample, at day 4). Although malondialdehyde levels increased over time, both 1% and 2% active films proved to be effective against the control. The migration assay showed that the antioxidant compounds were effectively released from the films, while maintaining their antioxidant functionality. Regarding the physicochemical characterization, there were no significant differences in moisture content (~14-16%) or water solubility (~52-53%) among the films. However, the swelling degree showed to be significantly decreased with the increase of extract concentration (from 35.1% in the control to 9.26% in the 2% extract film), suggesting a potentially higher water resistance. Opacity and transparency showed to be slightly higher for the 1% extract films, comparing to the control.

In conclusion, the incorporation of CIS extract for the production of active biodegradable films represents a promising alternative to the conventional unsustainable plastics, contributing to guarantee food safety, food security, and environmental responsibility through the principles of circular economy.

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From forest to packaging: Innovative phytosanitary strategies to support cork oak vitality

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Cork is a natural material of reference within the sustainable packaging industry, valued for its renewable origin, biodegradability, and technical performance in applications such as wine stoppers, seals, and insulation materials. Its quality and availability are closely linked to the health of the cork oak (*Quercus suber* L.), a species of major ecological and economic importance in the Mediterranean region, and particularly in Portugal,[1] where it underpins one of the country's most emblematic bio-based industries. In recent years, climate change has intensified environmental stress on cork oak stands, favoring the incidence of fungal diseases that can affect tree vitality and, consequently, the productivity and quality of the cork raw material. Among the pathogens of greatest interest are *Biscogniauxia mediterranea*, the causal agent of charcoal canker, and *Diplodia corticola*,[2,3] responsible for canker formation and progressive dieback. Both species act as latent endophytes, colonizing host tissues asymptomatically before triggering disease under stress conditions, which makes their detection, monitoring, and control particularly challenging.

The main objective of this work was to establish and validate an experimental framework for assessing the antifungal activity of different biocidal agents against these two pathogens, with the aim of supporting the development of even more effective and environmentally friendly phytosanitary treatments for cork oak protection. Given the endophytic nature of the fungi under study, and particularly the difficulty of obtaining viable spore material from *B. mediterranea*, considerable effort was devoted to optimizing fungal growth conditions prior to biocide testing.

Antifungal activity of the biocidal agents was evaluated using photodynamic activity. A spore-rich suspension of each study species were inoculated with the biocidal agents, with a pre-incubation time of 30 minutes to 5000 $mg.L^{-1}$ pyrogallol (PYR), 500 $mg.L^{-1}$ graphene oxide (GO) or 30.6 $mg.L^{-1}$ of Toluidine Blue O (TBO), followed by irradiation with LED-delivered white light (120 $mW{\cdot}cm^{-2}$) for 30 minutes. After this, the spore suspensions were inoculated in triplicate on DCRBA at 25º C during 3 or 5 days for *B. mediterranea* and *Diplodia corticola* respectively. The direct action of the biocidal agents was also tested to assess the cytotoxic effect of the compound against the fungal agents.

The data were analyzed using two-way ANOVA. In parallel, in vitro assays were conducted to obtain reliable spore suspensions from both species, and essential step for future dose-response and field-oriented studies.

Among the biocidal agents tested, compounds with a polyphenolic structure stood out, showing an average inhibition of 100% for both *D. corticola* and *B. mediterranea* at a concentration of 5000 $mL.L^{-1}$ PYR and 500 $mL.L^{-1}$ GO, demonstrating promising antifungal activity against both pathogenic species. In contrast, TBO at 30.6 $mL.L^{-1}$ showed 0% inhibition against *D. corticola* and *B. mediterranea*, indicating limited effectiveness under these conditions.

These results highlight their potential not only as direct phytosanitary agents but also as candidate building blocks for the future development of protective coatings that could be applied directly to cork oak trees following cork extraction and pruning, moments known to increase susceptibility to fungal infection. Such coatings would complement existing forest management practices, reinforcing the natural defense mechanisms of the tree while reducing reliance on conventional pesticides.

Overall, the methodology developed proved suitable and reproducible for evaluating antifungal efficacy under controlled laboratory conditions, yielding encouraging phytosanitary control results for both *B. mediterranea* and *D. corticola*. These findings represent a promising step toward reinforcing sustainable forest management practices for cork oak stands, contributing to the long-term preservation of tree health and to the consistent supply of high-quality cork. Ultimately, this work supports cork's position as a strategic, renewable resource for sustainable packaging solutions.

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Evaluation of antimicrobial activity of chitosan–alginate coatings incorporated with rosemary extract

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Growing environmental concerns about plastic packaging have led to increased interest in sustainable alternatives for food protection. In this context, biopolymer-based coatings have gained attention due to their biodegradability and film-forming properties. Furthermore, the incorporation of natural extracts into these systems will improve their antimicrobial potential and contribute to food preservation.1 This study aimed to develop a chitosan-alginate-based food coating incorporated with rosemary extract followed by its antimicrobial activity evaluation against food spoilage microorganisms.

Rosemary extract was obtained by hydroethanolic (80:20, v/v) maceration. Film-forming systems were prepared using a 1:1 mixture of 1% chitosan (90% D.D.) and 1% sodium alginate, with the rosemary extract being incorporated at 5 mg/mL. The effect of polymer combination together with rosemary extract, on antimicrobial activity was tested using the solutions and the following films: pure alginate, pure chitosan, chitosan-alginate mixture without extract, and chitosan-alginate mixture added of rosemary extract. The antimicrobial activity was assessed by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays, by applying two distinct methods: (i) for solution the serial microdilution method was used; while (ii) disk diffusion method was employed for films. In both methods the different samples were tested against several foodborne and spoilage microorganisms, including Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Yersinia enterocolitica, Bacillus cereus, Listeria monocytogenes, and Staphylococcus aureus.

The results obtained showed that alginate alone did not exhibit any antimicrobial activity, as expected, with MIC and MBC values above the maximum tested concentration for all microorganisms. This is related with alginate structural role without antimicrobial effect. In contrast, pure chitosan exhibited pronounced antimicrobial activity, with MIC values ranging from 0.3125 to 1.25 mg/mL and MBC values from 2.5 to 5 mg/mL. The highest sensitivity was observed for Y. enterocolitica and S. enterica. Disk diffusion assays also showed inhibition halos measuring 25 mm for S. enterica, 23 mm for L. monocytogenes, and 29 mm for S. aureus. This effect is related with the interaction of the protonated amino groups from chitosan with the negatively charged bacterial membranes.1 The antimicrobial activity of the chitosan-alginate mixture without rosemary extract was lowered, when compared to pure chitosan, pointing the polyelectrolyte complex formation as responsible for the reduction of free amino groups content, limiting the antimicrobial activity of chitosan.2 The incorporation of rosemary extract improved the antimicrobial activity of the chitosan-alginate system, resulting in lower MIC values for different microorganisms, particularly S. aureus (1.25 mg/mL) and S. enterica (2.5 mg/mL). Disk diffusion assays confirmed these results, showing inhibition halos mainly against S. enterica, L. monocytogenes, and S. aureus. These results point out the efficacy of the rosemary extract incorporation while antimicrobial enhancer of the chitosan-alginate system. Therefore, this study evidences the potential of chitosan-alginate coatings enriched with rosemary extract, for the development of bioactive food preservation systems. representing a safer, sustainable, and environmentally friendly packaging technology for the food industry.

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Mechanochemical modification of potato starch with spent frying oil for water-tolerant bioplastics

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Secondary raw materials generated during food industry processing, typically regarded as low-value by-products, can be valorized through the development of water-tolerant biodegradable bioplastics1. In this context, this study investigated how mechanochemistry promotes transesterification between the hydroxyl groups of starch and the fatty acyl chains of the triacylglycerides present in spent frying oil (SFO), using potassium hydroxide (KOH) as catalyst. The experimental approach evaluated the transesterification reaction and subsequent film-forming capacity of starch recovered from potato chips, using commercially available potato starch as a reference. Both were processed as native dry starch (with the recovered starch previously freeze-dried and sieved) and pregelatinized starch, the latter prepared as a strategy to promote granule disruption and increase hydroxyl group accessibility for chemical modification. The two starches exhibited a similar total starch and amylose content, both exhibiting high total starch contents (>95%) and amylose contents of 20%, respectively. When compared to neat starch-based films, all films with SFO/KOH exhibited improved stretchability and elasticity (both ca. 1–2×), and hydrophobicity (water contact angles of ca. 90º). Notably, films prepared from recovered pregelatinized starch exhibited higher hydrophobicity, reaching water contact angles of ca. 120º. ATR-FTIR spectra further confirmed the chemical modification of the starch samples through the incorporation of fatty acyl chains from SFO, as evidenced by structural differences of the carbonyl band (1740 cm-1) among the produced films, although further studies are required to better understand the extent of this modification. Overall, these findings provide a foundation and highlight a promising green strategy for the development of water-tolerant starch ester-based bioplastics with enhanced hydrophobicity while simultaneously valorizing secondary raw materials from potato industry processing.

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Valorization of Lobosphaera sp. microalgae biomass in the production of biodegradable bioactive films for strawberry preservation

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The short shelf life of fruits like strawberries and the environmental impact of conventional plastic packaging motivate the development of biodegradable and active packaging from natural resources. Microalgae constitute an eco-innovative marine biomass, rich in bioactive macro and micronutrients, with potential for the production of functional edible films. This work aimed to valorize the biomass of *Lobosphaera* sp., through the extraction of bioactive compounds, polysaccharides, and protein, incorporating them into alginate films, and evaluating their physicochemical, antioxidant and antimicrobial performance, as well as their effectiveness in the post-harvest preservation of strawberries.

From the biomass of *Lobosphaera* sp. extracts rich in polysaccharides (PS), protein (P) and bioactive compounds were obtained and used in the formulation of three films by casting: A – 2% alginate (control); B – alginate (2%) + PS extract (0.5%) + bioactive extract (0.25%); C – alginate + P extract (0.5%) + bioactive extract (0.25%). The films were characterized for antioxidant activity (ABTS, DPPH), physical properties (solubility, water vapor permeability, elongation at break, and tensile strength), and antimicrobial activity (growth curves of *Listeria monocytogenes* and *Salmonella enterica* Serovar Enteritidis). The films were also applied to the packaging of strawberries, evaluating water loss and microbial load (aerobic mesophilic bacteria, molds, and yeasts) throughout storage.

The incorporation of extracts into alginate films did not compromise mechanical performance (no statistically significant differences in elongation at break and tensile strength between formulations, $p > 0.05$), while antioxidant activity increased markedly with bioactive enrichment, reaching 451 µM TE/mg of film (ABTS) and 213 µM TE/mg of film (DPPH) in formulation C, values 2.5 to 4 times higher than the control. All three films showed 100% water solubility, with the film with protein-rich extract (C) showing less water vapor permeability than the control. The enriched films (B and C) also retarded the growth of L. monocytogenes compared to the control. When applied to strawberries, these films reduced water loss and microbial contamination (mesophilic bacteria, molds, and yeasts) during storage, compared to the control film.

Lobosphaera sp. biomass constitutes a valuable source of bioactive compounds that can be used in biodegradable edible films with enhanced antioxidant and antimicrobial properties, capable of safely extending the post-harvest shelf life of strawberries. These results reinforce the potential of microalgae in the design of active packaging within the framework of a circular economy.

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Design of Sustainable Cellulose Absorbent Pads with Grape Stem Extract for Active Food Packaging: Bioactive Compound Release and Impact on the Sensory Quality of Chicken Meat

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Grape stems, an abundant by-product of the wine industry, represent a valuable source of phenolic compounds with well-documented antioxidant properties. Their incorporation into cellulose absorbent pads offers a sustainable strategy for the valorisation of agro-industrial residues while providing an active packaging system with the potential to release bioactive compounds. In this context, this study aimed to develop cellulose absorbent pads containing grape stem extract (GSE) and evaluate their colour characteristics, the migration of phenolic compounds and antioxidant activity under food simulant conditions, as well as their effect on the sensory quality of refrigerated chicken meat.

Grape stem extract (GSE) was obtained by ultrasound-assisted extraction (4 g/20 mL, ethanol:water 50:50, 15 min, 25 °C), followed by solvent removal and freeze-drying. Absorbent pads were produced from microcrystalline cellulose using a NaOH/glycerol system, resulting in final cellulose, NaOH, and glycerol concentrations of 5, 8, and 0.5 g/100 g, respectively. The pads were enriched with GSE by surface application (0.5 g/pad), followed by storage at 4 °C for 24 h. Subsequently, the pads were washed to remove non-incorporated extract residues and dried at 25 °C for 18 h. Four pad formulations were developed: microcrystalline cellulose (MC), microcrystalline cellulose containing grape stem extract (MC-GSE), microcrystalline cellulose coated with ethyl cellulose (MC-EC), and microcrystalline cellulose containing grape stem extract and coated with ethyl cellulose (MC-GSE-EC). Surface colour was determined according to the CIELab colour space (L*, a* and b*) and the colour difference (ΔE) was calculated. Migration assay was determined using Payne permeability cups (10 cm2), filled with 12 mL of 10% ethanol as the food simulant. The pads were placed in direct contact with the simulant and maintained at 4 °C, and analysed at 24 h and 9 days. The release of bioactive compounds was assessed by determining the total phenolic content (Folin-Ciocalteu method) and antioxidant capacity (ABTS●+ scavenging capacity). The performance of the developed pads was further evaluated during refrigerated storage of fresh chicken meat. Chicken breast samples (≈20 g) were packaged in plastic trays with pads, sealed with film, and stored at 4 °C for 9 days. Sensory quality was assessed by quantitative descriptive analysis (QDA) with 6 semi-trained sensory panel with a background in meat quality. The panel evaluated visual appearance, odour and overall freshness using a structured 9-point intensity scale.

The release of phenolic compounds from MC-GSE pads into 10% ethanol increased progressively throughout storage. Total phenolic content increased from approximately 230 to 780 mg GAE/L, while antioxidant activity increased from 1.2 to 3.9 mM Trolox equivalents after 9 days, demonstrating a sustained release of bioactive compounds. The incorporation of GSE significantly affected the colour of the absorbent pads. Compared with MC pads, MC-GSE exhibited a marked reduction in lightness (L*), and a significant increase in redness (a*) and yellowness (b*). These changes are attributed to the characteristic pigmentation of GSE. Regarding sensory quality, MC-GSE maintained characteristic odour scores comparable to the control throughout refrigerated storage, indicating that grape stem extract did not negatively affect the sensory perception of chicken meat. Herbaceous odour remained negligible in all treatments.

The developed cellulose absorbent pads demonstrated the ability to provide sustained release of phenolic compounds while preserving their antioxidant activity under refrigerated conditions. The incorporation of GSE intensified the pad colour but did not adversely affect the sensory perception of chicken meat, highlighting the potential of grape stem extract as a sustainable bioactive ingredient for the development of active cellulose-based packaging systems.

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Towards sustainable rice packaging: Evaluating materials as alternatives to conventional plastic

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The environmental challenges associated with conventional multilayer plastic packaging have intensified the search for circular and bio-based alternatives for dry food applications, such as rice1. Current commercial packaging, typically based on biaxially oriented polypropylene (BOPP) laminated with polyethylene (PE), provides excellent mechanical strength, seal integrity, and moisture barrier performance2; however, its fossil-based origin and limited recyclability are inconsistent with the objectives of Regulation (EU) 2025/40 on packaging and packaging waste (PPWR), which requires packaging systems to become fully recyclable and structurally simplified by 2030. In this context, paper-based and cellulose-derived materials have emerged as promising candidates for sustainable packaging redesign.

This review evaluates kraft paper films and NatureFlexTM cellulose films (30 and 45 µm) as potential alternatives to conventional plastic rice packaging. It critically compares their mechanical performance, barrier properties, recyclability and expected ability to preserve rice quality during storage. Particular attention is given to the need for functional coatings to improve barrier properties and heat sealing, and to how these modifications may affect circularity and compliance with PPWR requirements.

An additional aspect considered in this review is resistance to Sitophilus spp. infestations, one of the most important storage pests of rice. The susceptibility of kraft paper and NatureFlexTM films to insect penetration and perforation is considered, together with the influence of material structure and film thickness on pest deterrence and package integrity during storage. By comparing these bio-based materials in terms of functional performance, environmental impact, regulatory alignment, and resistance to biological hazards, this analysis identifies the main advantages, limitations and research gaps highlighting their potential contribution to sustainable rice packaging.

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Exploring the potential of SCOBY bacterial cellulose films in Green Tea Kombucha to produce food packaging

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Cellulose is the most abundant polymer on Earth and represents an inexhaustible source of material for diverse applications, from cosmetics, biotechnology, textiles, processing and the medical field, due to its biocompatibility1,2. Bacterial cellulose, nanofibrillar material produced by microbial fermentation, offers superior purity, water retention, and mechanical properties.

In kombucha fermentation, a symbiotic culture of bacteria and yeast (SCOBY) produces bacterial cellulose films as a natural by-product. These films exhibit high surface area and notable resistance to aqueous environments, excellent biocompatibility and non-toxicity (characteristics that make them attractive candidates for food packaging, biomedical applications, particularly wound dressings)3.

This study focused on the production of bacterial films using green tea kombucha, under different conditions: inoculum preparation, fermentation temperature, and drying method. The resulting films were characterized using FTIR-ATR, water contact angle measurements, water vapor transmission rate, and additional techniques. A scheme of the process is shown in Figure1.

Preliminary results revealed the formation of multilayered films derived from SCOBY. Films fermented at ambient temperatures (15-18ºC) exhibited fewer layers compared to those incubated at elevated temperatures (38ºC). Additionally, the films were capable of absorbing more than 100% of their own weight in water. Based on their structural and physicochemical properties, the most promising films for food packaging applications were identified.

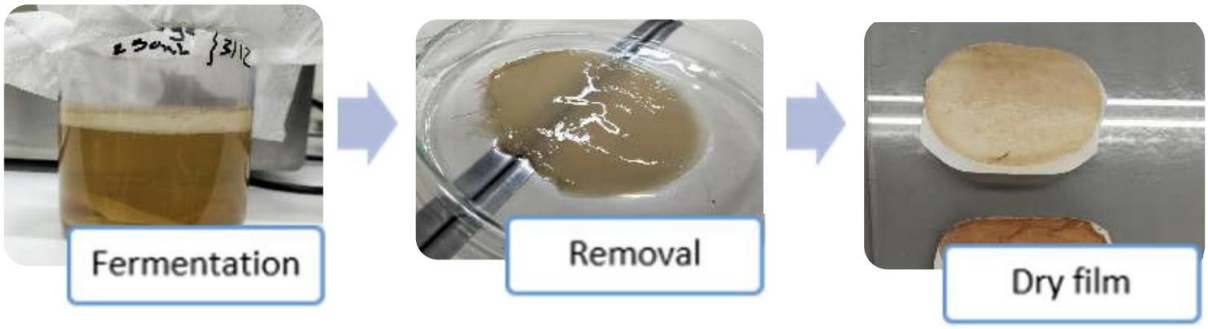


Figure 1 - Formation of bacterial cellulose films

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Development of Active and pH-Responsive Pullulan and Agar Films Incorporating Pomegranate Bioactives for Food Packaging Applications

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The development of sustainable food packaging materials with active and intelligent functionalities is a key strategy to improve food quality and reduce environmental impacts. In this work, films based on pullulan and agar were developed incorporating freeze-dried pomegranate (Punica granatum L.) extract, a natural source of phenolic compounds and anthocyanins. The pomegranate extract exhibited a high total phenolic content (1559 ± 7 mg GAE/100 g) and contained several bioactive compounds, including ellagitannins, flavonoids and anthocyanins such as cyanidin and delphinidin derivatives.

The influence of pomegranate incorporation on the physicochemical, thermal, antioxidant and intelligent packaging properties of pullulan and agar films was evaluated. The incorporation of pomegranate increased film thickness and modified mechanical behavior, particularly in pullulan films, which showed a substantial increase in flexibility and folding endurance. Enhanced water barrier properties were observed for both polymer matrices, with water vapor permeability decreasing for pullulan and agar films. Antioxidant activity was also imparted to the films, reaching 15.84% of DPPH scavenging activity and 70.78% inhibition in the β-carotene bleaching assay for agar-based formulations.

Thermogravimetric analysis revealed that pomegranate incorporation introduced an additional degradation stage between 150 and 220 °C and promoted the formation of a larger carbonaceous residue during thermal degradation, suggesting interactions between the bioactive compounds and the polymer matrices. Differential scanning calorimetry showed no significant thermal transitions below the degradation temperature, indicating the preservation of the amorphous structure of the films.

Furthermore, pomegranate-loaded films exhibited remarkable pH-responsive color changes over a wide pH range (1-14), associated with anthocyanin structural transformations. This behavior demonstrates their potential as intelligent indicators for monitoring food freshness and quality.

In conclusion, pullulan and agar films incorporating pomegranate bioactives exhibited antioxidant activity, enhanced barrier properties, and a pH-sensitive colorimetric response, demonstrating their potential as sustainable active and intelligent packaging materials for food applications.

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XVIII Encontro de Química dos Alimentos

Coimbra | 14 - 16 de outubro'26

DA INVESTIGAÇÃO AO MERCADO: CIRCULARIDADE E CRIAÇÃO DE VALOR ECONÓMICO



Licenciada em Química Tecnológica pela Faculdade de Ciências da Universidade de Lisboa e exerce, desde 2013, funções como Gestora de Investigação Aplicada na Sumol+Compal Marcas, S.A. Nesta função, é responsável pela gestão da interface entre a empresa e a Academia, bem como pelo acompanhamento de projetos de investigação e inovação cofinanciados. Iniciou a sua atividade profissional na Sumol+Compal em 1992, tendo desempenhado diversas funções nas áreas técnicas e de gestão. Ao longo do seu percurso profissional, tem desenvolvido atividade na articulação entre investigação aplicada, inovação e indústria alimentar, contribuindo para a aproximação entre o conhecimento científico e as necessidades do setor empresarial.

Ciência - Um Ingrediente para a Inovação

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A definição de Inovação é fundamental para melhor se compreender o papel da ciência na gestão da inovação.

A relação entre Academia e Indústria deve assentar num ambiente de conhecimento e confiança mútuos (com mecanismos que mitiguem enviesamentos), compreensão dos desafios (existem vários para a indústria alimentar) e proporcionar uma comunicação da ciência dos alimentos que confira confiança aos vários stakeholders.

O caso Sumol Compal na aplicação de C&T no processo de inovação evidencia a virtuosidade da relação com a Academia.

Figure 1 - Do núcleo ao sistema: a química dos alimentos como ciência em camadas.

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Pear Pomace as a Sustainable Feedstock for Sugar Syrup Production: Physicochemical and Bioactive Characterization Supporting Circular Valorization

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INTRODUCTION: Pear processing generates large amounts of pomace, which is commonly discarded despite containing valuable nutrients and bioactive compounds. Within the framework of the circular bioeconomy, the recovery of this by-product represents an opportunity to develop innovative food ingredients while reducing food waste. Due to its natural sugar content, dietary fiber and antioxidant compounds, pear pomace may constitute an attractive raw material for the production of naturally derived sugar-rich syrups.

OBJECTIVE: This study aimed to characterize pear pomace in terms of its nutritional composition, sugar profile, mineral content and bioactive properties to evaluate its suitability as a feedstock for the production of a sugar-rich syrup through enzymatic hydrolysis.

METHODS: Pear pomace obtained from industrial juice processing was freeze-dried and characterized for proximate composition, dietary fiber and mineral content using AOAC official methods and ICP-OES, respectively. Individual sugars were quantified by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Total phenolic content was determined using the Folin–Ciocalteu assay, while antioxidant capacity was evaluated by ABTS and DPPH radical scavenging methods in free and bound phenolic extracts.

RESULTS: Pear pomace exhibited a carbohydrate-rich composition (95.63 g/100 g DM), containing 41.72 g/100 g DM of total sugars and 53.92 g/100 g DM of total dietary fiber, predominantly insoluble fiber. Sorbitol was the major soluble carbohydrate (21.51 g/100 g DM), followed by glucose (14.54 g/100 g DM) and fructose (8.99 g/100 g DM), indicating a favorable composition for producing sugar-rich ingredients. The pomace also contained appreciable levels of essential minerals, with potassium (675 mg/100 g DM) and calcium (101 mg/100 g DM) being the most abundant. Regarding bioactivity, free phenolic extracts showed a total phenolic content of 3.68 mg GAE/g DM, while alkaline-bound phenolics reached 6.59 mg GAE/g DM, highlighting the importance of bound phenolic fractions. Antioxidant capacity was confirmed by both ABTS and DPPH assays, with the highest values observed in free phenolic extracts (10.01 and 6.72 mg TE/g DM, respectively), demonstrating that pear pomace retains bioactive compounds after juice processing.

CONCLUSION: The high concentration of fermentable sugars, combined with significant dietary fiber, mineral content and antioxidant potential, demonstrates that pear pomace is a promising raw material for the development of value-added food ingredients. These findings support its valorization through the production of sugar-rich syrups, contributing to waste reduction, resource efficiency and the implementation of circular economy strategies in the fruit processing industry.

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Unveiling the biorefinery concept from brown seaweed: a green cascade strategy to obtain multifunctional fractions

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The interest in seaweeds have grown significantly over the years and they are recognized as valuable feedstocks for the bioeconomy, due to their high nutritional value and unique biocompounds with several industrial applications, as well as their biorefinery potential.[1] Sustainable extraction processes, such as subcritical water extraction, are alternatives to traditional extraction due to high extraction efficiency and environmental benefits. Subcritical water extraction is a hydrothermal treatment based on water's subcritical properties, operating at temperatures and pressures above the boiling point of the water.[2]

The aim of this work is to explore the full potential of brown seaweed *Fucus vesiculosus* valorization, based on a biorefinery model, to sequentially extract multiple bioactive compounds (e.g. pigments, phenolics, proteins, alginate, fucoidan) with several functionalities, performing cold aqueous, ethanolic, alkaline and subcritical water extraction, in a more efficient strategy.

Extraction experiments were performed according to the following sequence: (i) cold aqueous extraction at room temperature for 24 h; (ii) ethanolic extraction using 96 % ethanol at room temperature for 24 h in darkness; (iii) alginate extraction with 1 M Na_2CO_3 at 60 °C for 90 min and (iv) fucoidan extraction using a stirred pressurized reactor at 170 °C for different processing times (1 s, 1 min, 10 min and 17.5 min) with subcritical water; conventional fucoidan extraction (90 °C for 240 min) was carried out as a control. The different extracts obtained were analyzed for their chemical composition, texture profile, bioactive potential and cytotoxicity.

The results showed that the ethanolic extraction resulted in a pigments content of 1.3 % (fucoxanthin accounting for 0.8 %), phenolic compound yield of 26.3 mg GAE/g extract DW with good antioxidant activity. *Fucus*'s alginate displayed interesting palatable qualities and good physical properties. All fucoidan-rich fractions exhibited high content of the characteristics sugars, L–glucuronic acid (54.5–59.0 g/100g) and fucose (12.5–20.7 g/100g), and a good capacity to promote the growth of probiotic bacteria such as *Lactobacillus* species, indicating that there is some potential of prebiotic effect. *Fucus*'s extracts showed a great foaming (76.2–123.8 %) and emulsifying stability (65–95 %), mostly due to their content in protein (12 %), which confirm that these extracts can be applied in the development of new food products, specifically the fraction at 1 min of processing. The supernatant fraction obtained after fucoidan precipitation exhibited promising results of antibacterial activity against gram-positive bacteria, particularly against pathogenic *S. aureus* and *B. subtilis*. All extracts obtained using subcritical water extraction were considered non-toxic, i.e., safe for human consumption.

Therefore, this green extraction technique demonstrated to be highly efficient by improving extraction efficiency, reducing processing time, while facilitating the production of high-value products. Additionally, this strategy proved the viability of valorizing different functional fractions. These findings allow the contribution to the development of sustainable biorefinery models for *Fucus vesiculosus*, enhancing their potential for commercial applications in high-value industries.

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From insect feeding to healthy foods: DHA enrichment of Acheta domesticus using Schizochytrium sp. and application in a healthy snack bar

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The increasing demand for sustainable protein sources has intensified interest in edible insects as alternative foods capable of supporting future food security. Among these, *Acheta domesticus* has emerged as a promising candidate due to its high nutritional value, efficient feed conversion, and comparatively low environmental footprint. Moreover, the nutritional composition of insects can be strategically modified through dietary interventions, allowing the production of value-added ingredients with improved health benefits.

This study aimed to enhance the nutritional quality of *A. domesticus* through dietary supplementation with the DHA-rich (docosahexaenoic acid, 22:6n-3) marine microalgae *Schizochytrium* sp. and to evaluate the potential of the resulting cricket flour for the development of a functional snack bar.

Crickets were reared on three experimental diets: a standard diet (CT), and diets supplemented with 5% or 10% *Schizochytrium* sp. The total lipids content, lipid classes profile and fatty acid composition were subsequently determined in the obtained cricket flours as well as in the functional snack bar.

Total lipid content of the cricket flours ranged from 21.2 ± 1.5 to 23.7 ± 1.9% (dry weight) and was not significantly affected by dietary treatment. Among the lipid classes non-polar lipids predominated, with triacylglycerols (TAG) accounting for approximately half of the total lipid fraction, while the highest TAG proportion was observed in crickets receiving the 5% supplemented diet.

Concerning the fatty acids profile, palmitic (16:0), linoleic (18:2n-6) and oleic (18:1n-9) acids, stood out as predominant. Importantly, docosahexaenoic acid (DHA), which was absent in the control group, increased progressively with microalgae inclusion, reaching 2.8 ± 0.4% of total fatty acids in crickets fed the 10% supplemented diet. These findings demonstrate that dietary supplementation increased DHA deposition without affecting the overall lipid content of the insects.

To demonstrate the potential application of the enriched flour, a snack bar was subsequently formulated using the flour obtained from crickets fed with 10% *Schizochytrium* sp. in combination with other nutritionally valuable ingredients, including egg white, cocoa powder, dates and peanut butter, targeting consumers seeking healthy and organic snack alternatives. The developed product presented a proximate composition of 21.9 ± 0.6% moisture, 15.7 ± 0.1% protein, 8.9 ± 0.1% of total lipids and 2.6 ± 0.1% ash. Among lipids, 18:1n-9, 18:2n-6 and 16:0 were the most relevant fatty acids.

Despite dietary supplementation with *Schizochytrium* sp. proved to be a promising strategy for enhancing the nutritional quality of edible insects, the present findings indicate that there is still potential to improve the efficiency of DHA incorporation into *A. domesticus*. Further optimization of this approach may enable more consistent DHA enrichment, supporting the use of insect-based foods as sustainable complementary sources of long-chain omega-3 fatty acids. Besides, the successful incorporation of the enriched flour into a ready-to-eat snack highlights the potential of nutritionally tailored edible insects as sustainable functional food ingredients and supports the development of innovative omega-3-enriched insect-based products for human consumption.

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Granulometric Fractionation as a Tool to Enhance the Functional Value of Apple Pomace

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Apple pomace is one of the major by-products generated by the fruit juice industry, accounting for approximately 25–30% of processed apple mass.[1] Although traditionally used as animal feed or fertilizer, its rich composition in dietary fibre and bioactive compounds makes it an attractive raw material for the development of functional food ingredients.[2] This work aimed to evaluate the effect of granulometric fractionation on the chemical composition and bioactive potential of industrial apple pomace flour.

Dried apple pomace obtained from industrial juice production was milled and separated into five particle-size fractions (>1 mm, 710 µm, 180 µm, 75 µm and 53 µm). The fractions were characterized using complementary chromatographic approaches, including GC-MS for lipophilic compounds, UPLC-PDA for phenolics and carotenoids, UPLC-FLR for tocopherols and tocotrienols, and UPLC-PDA/MS for free amino acids. Antioxidant capacity was assessed through ABTS and ORAC assays, while acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities were evaluated as indicators of potential neuroprotective properties.

The results demonstrated a strong influence of particle size on the distribution of bioactive compounds. The 180 µm fraction exhibited the highest concentration of total phenolic compounds (1013 mg/100 g dw), flavonoids (1008 mg/100 g dw), polymeric procyanidins (716 mg/100 g dw) and carotenoids (50 mg/100 g dw). Antioxidant activity was significantly higher in the 710 and 180 µm fractions, reaching 2.8 mmol Trolox/100 g dw (ABTS) and 6.8 mmol Trolox/100 g dw (ORAC). Distinct distributions were also observed for vitamin E compounds, with total tocopherols and tocotrienols reaching up to 886 µg/100 g dw, and for free amino acids, where all nine essential amino acids were identified.

Interestingly, selected fractions exhibited relevant anticholinesterase activity. The 710 µm fraction showed the highest inhibition of AChE (27.5%) and BuChE (24.3%), while the 53 µm fraction also demonstrated strong inhibitory activity. These findings suggest that specific apple pomace fractions may provide bioactive compounds capable of modulating cholinergic pathways associated with cognitive health and ageing.

Overall, the results demonstrate that granulometric fractionation represents a simple, sustainable and low-cost strategy for obtaining apple pomace ingredients with differentiated compositional and functional properties. The fraction-specific enrichment of phenolics, carotenoids, vitamin E isoforms and neuroprotective-related activities highlights the potential of apple pomace as a valuable source of next-generation functional ingredients, supporting both circular economy principles and the valorisation of agro-industrial residues.

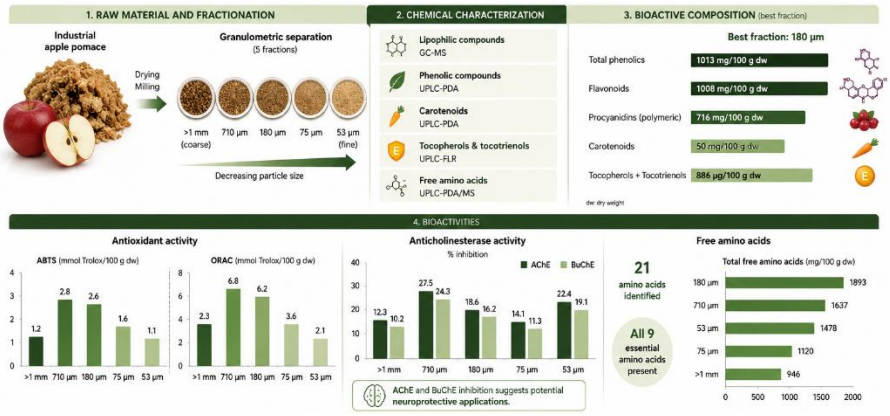


Figure 1 - Overview of the granulometric fractionation of apple pomace flour and its impact on chemical composition and bioactive properties.

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Agar extraction solid residue as a source of texturing polysaccharides with bioactive properties

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Agar is a sulfated polysaccharide extracted from Gelidium spp. and is widely used for its gelling and thickening properties. However, Industrial agar production generates a solid residue containing 32% carbohydrates (16% glucans and 16% agar), 33% ash, 18% protein, and 9% moisture, which remains largely undervalued[1]. This work explores the valorization of this by-product through the extraction and characterization of bioactive polysaccharides.

Hot-water extraction (95 °C, 1–4 h) demonstrated that a 2 h treatment was sufficient for efficient carbohydrate recovery. The extract consisted mainly of galactose, 3,6-anhydrogalactose, and sulfate esters, confirming the presence of agar-derivatives. Cellulase pretreatment increased the extraction yield without affecting the overall monosaccharide composition.

The extract exhibited a total phenolic content of 28 µg GAE/mL, determined by the Folin–Ciocalteu method, significantly higher than that of commercial agar (4 µg GAE/mL). Consistent with this result, it exhibited enhanced antioxidant activity in the ABTS assay, with an IC_{50} value of 0.8 mg/mL, whereas commercial agar showed no detectable inhibition at concentrations up to 2 mg/mL. In a glycodeoxycholic acid micellar intestinal model, the extract also showed the capacity to reduce cholesterol solubility by 45%, indicating potential hypocholesterolemic activity. These findings suggest that, in addition to agar, the extract retains considerable amounts of others bioactive compounds.

Rheological properties were assessed through frequency-sweep measurements. The storage modulus (G') exceeded the loss modulus (G''), confirming the gel-like behavior of the extract. Although its gelling strength was lower than that of commercial agar, the extract displayed higher G' values than potato starch, indicating promising thickening and gelling potential.

Overall, these results highlight Gelidium spp. processing residues as a valuable source of functional polysaccharides with antioxidant, hypocholesterolemic, and rheological properties, supporting their potential application as food ingredients and contributing to the sustainable valorization of marine biomass within a circular bioeconomy.

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From feed to food chemistry: Effect of olive cake supplementation on the physicochemical, lipid and volatile profiles of traditional Bísaro dry-cured products.

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The growing demand for sustainable food production has promoted the valorization of agro-industrial by-products within a circular economy framework. Olive cake, the main by-product of olive oil extraction, is produced in large quantities in Mediterranean countries and contains fibre, residual lipids rich in oleic acid, and bioactive compounds with potential nutritional value. Its incorporation into livestock diets represents a sustainable alternative to conventional feed ingredients, contributing to waste reduction while potentially influencing the chemical composition of animal-derived foods. The Bísaro pig, an autochthonous Portuguese breed used in the production of traditional dry-cured products, provides an excellent model to evaluate how olive cake supplementation may affect the chemical characteristics and quality of traditional meat products.

The present work provides an integrated overview of experimental studies evaluating the effects of incorporating olive cakes obtained by different olive oil extraction systems into the finishing diets of Bísaro pigs. Rather than focusing on a single product or analytical parameter, this communication adopts a “feed-to-food-chemistry” perspective, integrating the main findings obtained for several traditional dry-cured products, including loin, “cachaço” and shoulder. The impact of olive cake supplementation was assessed through physicochemical characterization, lipid composition, fatty acid profiling, volatile compound determination, and sensory evaluation. Overall, the incorporation of olive cake into the diets of Bísaro pigs did not adversely affect the physicochemical quality of the dry-cured products. Parameters such as pH, moisture, protein, intramuscular fat, and ash content remained within the characteristic ranges expected for these traditional products, indicating that the replacement of conventional feed ingredients with olive by-products did not impair the technological properties associated with curing and ripening processes. The analysis of lipid composition showed that olive cake supplementation preserved the characteristic fatty acid profile of Bísaro dry-cured products. MUFA, particularly oleic acid, remained the predominant fraction, followed by SFA and PUFA. The inclusion of olive cake did not induce detrimental changes in the nutritional quality commonly associated with the health value of meat products. This stability suggests that the use of olive cake can contribute to the sustainability of production systems while maintaining lipid quality attributes valued by consumers and the meat industry. Volatile compounds were also investigated as key indicators of aroma development and overall product quality. The integrated analysis of the volatile profile revealed that dietary olive cake incorporation did not promote undesirable modifications in aroma-related compounds during curing. The major chemical families identified, including aldehydes, alcohols, ketones, and hydrocarbons, were consistent with those typically described for traditional dry-cured pork products. Sensory assessment further demonstrated that chemical changes were not associated with negative impacts on the organoleptic characteristics of the products, confirming the technological suitability of this feeding strategy (Leite et al., 2022; Leite et al., 2023; Leite et al., 2023a)

From a food chemistry perspective, these results highlight the close relationship between animal feeding practices and the chemical composition of the final food product. The valorization of olive oil by-products through their incorporation into pig diets can therefore be integrated into traditional meat production chains, supporting circular economy principles while preserving the physicochemical, lipidic and aromatic characteristics that define product quality and authenticity.

In conclusion, olive cake supplementation represents a viable strategy for linking agro-industrial by-product valorization with the production of high-quality traditional foods. The integrated evidence indicates that this ingredient does not compromise the physicochemical, nutritional, or sensory attributes of dry-cured products, reinforcing the potential sustainable feeding practices to support resilient and environmentally responsible food systems.

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Impact of ripening on the sugar profile and total phenolic content and antioxidant activity from substandard or defective fruit: apple and pear

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Fruit is one of the essential foods in human nutrition, with apples and pears being the most produced fruits in Portugal. In the period 2018-2023, the average percentage of defective fruit and substandard in the total production of the Portuguese FRUTUS- SPF – Fruit Production and Marketing Company, LTD. is, 8%, 10%, and 25% for PDO Rocha pear, PGI Gala apple, and PGI Fuji apple from Alcobaça, totaling 158 tons/year of pears and 138 and 296 tons of Gala and Fuji apples, respectively. Fruit with defects or small size, also called "fruta feia" by the industry, is considered processing-grade fruit, i.e., it is routed to the industrial sector for juices, jams, or purees, because it fails fresh-market visual standards. (Figure 1). The objective of this study was to evaluate the sugar content of this type of fruit with two different ripening stages and the total phenolic content and DPPH-assay values.



Figura 1. Examples of substandard or defective fruit.

Nutritional characterization and bioactive compounds quantification were performed on PDO Rocha pears, PGI Gala and PGI Fuji apples, including sugar profiles by HPLC-RI. Comparative tests were conducted between fruit stored for approximately 180 days in cold chambers with a nitrogen atmosphere and fruit that, after this storage, was placed for 20 days under normal atmosphere conditions and an average temperature of 20°C, identified as over-ripeness. Sugar quantification was performed using HPLC-RI. The total sugar concentration shows a decrease in ripe fruit of 11% for Fuji apples and 26% for Rocha pears. For Gala apples, there are no significant differences in total sugar concentration between the two ripening stages. In Gala and Fuji apples, there are significant differences between the two ripening stages in sucrose concentration, with lower values for over-ripeness. In Rocha pear, between the two ripening stages, there are no differences in sucrose concentration, but there are differences in glucose, fructose, and sorbitol concentrations, with lower values for over-ripeness (Figure 2). These results indicate that overripeness decreases the total sugar content in Fuji apple and Rocha pear, but not in Gala apple. The total fruit sugar content can indeed decrease once some fruit passes its peak ripeness and enters the over-ripeness stage. This process converts sugars into energy, and other plant compounds. Gala and Fuji apple have higher sucrose and glucose contents than Rocha pear (Figure 2). These results are promising for the application of Rocha pear and Fuji apple in glycemia regulation, combining the over-ripeness stage.

Phenolic compounds were extracted with water and quantified using the Folin–Ciocalteu method according to Singleton and Rossi (1965)[1] and Ainsworth and Gillespie (2007)[2]. The three types of fruit presented higher values for both TPC and antioxidant activity-DPPH assay in unripe fruit. These results are in accordance with a study in fruta-do-lobo (Solanum lycocarpum St. Hill)[3].

These data suggest that fruit can present different chemical compositions due to their ripening stage, providing support for future research aimed at the application of these fruits in glycemia control or the use of their extracts with a higher content of antioxidant compounds.

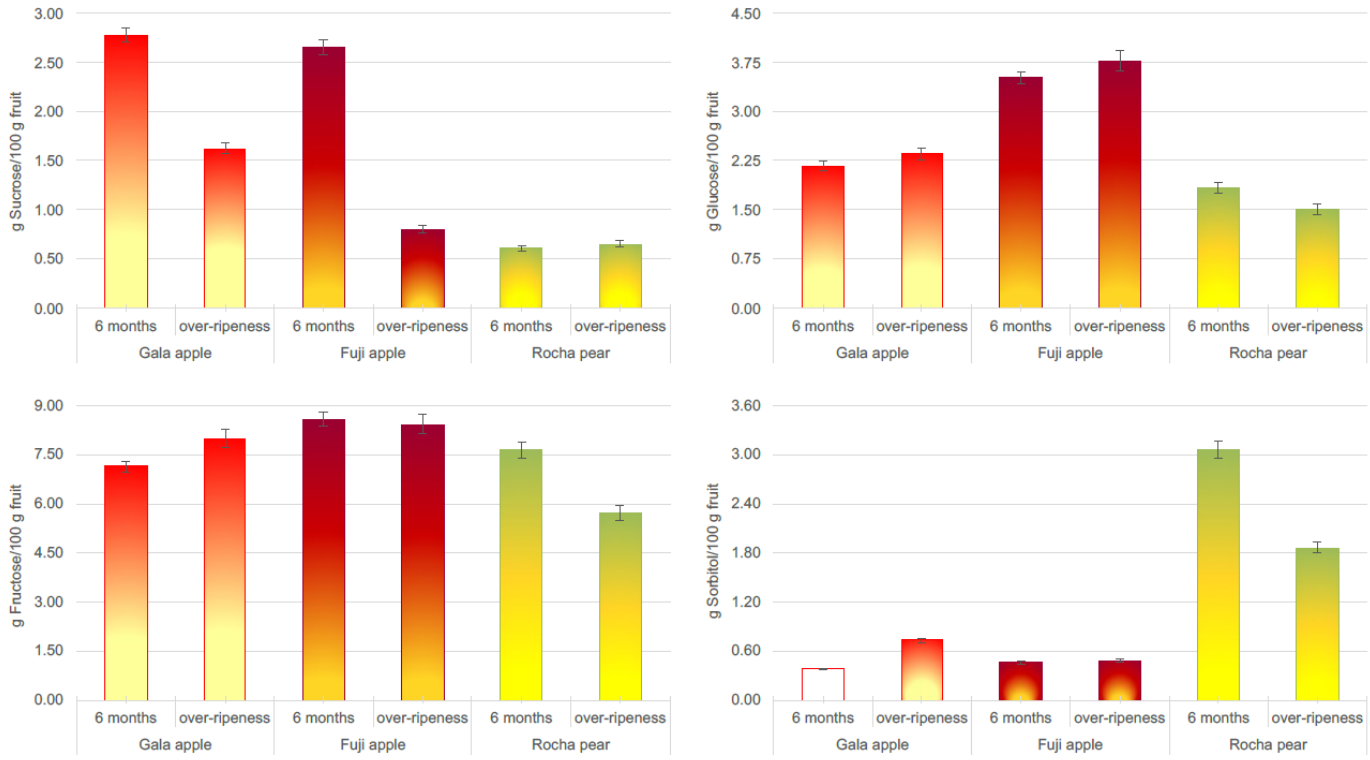


Figure 2 – Sugar profile in g/100g of fruit preserved for 6 months in a modified atmosphere cold chamber and fruit with over-ripeness.

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Structural characterization of polysaccharide rich fractions from Agaricus bisporus by-products and their cholesterol lowering potential

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Mushroom consumption has been increasing 10 % per year globally[1] due to their unique organoleptic properties and the rising demand for healthy, vegan and high protein foods. The global mushroom market size was around 20 ktons, generating approximately 60 ktons of by-products that are currently used as fertilizers or animal feed[2]. However, these by-products are a rich source of proteins and polysaccharides, particularly β-glucans which have been associated with the hypocholesterolemic potential[3] and represent a significant opportunity for valorization into high-value applications.

In this work, extraction and detailed characterization of different polysaccharide rich fractions from *Agaricus bisporus* by-products were addressed targeting its valorization as functional food ingredients.

Ethanolic, aqueous and alkaline extracts of *Agaricus bisporus* by-products resulted in low yield of soluble (19-29 %) and high yield of insoluble (54-56 %) polysaccharides. The fractions obtained were rich in carbohydrates (37-83 %), being glucose the major monosaccharide residue (44-96 mol%). From hot water extraction, the insoluble residue (WIR) that yielded 39 % of mushroom was composed by 28 % of protein and 53 % carbohydrates with 69 mol% of glucose and 19 mol% of glucosamine. The characteristic glycosidic linkage profile was (1→4)-Glc (30 mol%), (1→6)-Glc (23 mol%) and (1→4)-GlcNAc (11 mol%). The alkaline extraction with NaOH 1M was performed to the previous residue with and without reducing agent which prevents the β-elimination reactions preserving glucans. Comparing the results of both extractions, the supernatant of without reducing agent (Sn_1M.NaOH) yield 16 % of mushroom and was richer in carbohydrates (83 %) mainly glucose (96 mol%) than the supernatant with reducing agent (Sn_1M.NaOH_RA) that yield 5 % with 72 % of carbohydrates and 92 mol% of glucose. Without reducing agent, the soluble glucan was composed by (1→4)-Glc (43 mol%) and (1→6)-Glc (26 mol%) together with (1→3,4)-Glc (14 mol%) linkages in contrast with reducing agent that yielded mainly (1→4)-Glc (62 mol%) and (1→6)-Glc (14 mol%) linked glucans. Upon supernatant dialysis of both extractions, it was obtained a precipitate. Without reducing agent, the ppt_Sn1M.NaOH, yield 3 % from mushroom, was composed of 57 % of carbohydrates from which 94 mol% was glucose with (1→3)-Glc (39 mol%), (1→4)-Glc (24 mol%) and (1→3,4)-Glc (15 mol%) linked. The same fraction with reducing agent (ppt_Sn1M.NaOH_RA) yield 2 % and was richer in carbohydrates (77 %) mainly glucose (90 mol%) with (1→6)-Glc (40 mol%) followed by (1→4)-Glc (15 mol%) and (1→3)-Glc (13 mol%) linked. The final residue of both extractions shows similar yields (12-15 %). However, the obtained without reducing agent (R_1M.NaOH) was composed of 63 % of carbohydrates with 50 mol% of glucose and 45 mol% of glucosamine in contrast with the obtained with reducing agent that was composed of 39 % of carbohydrates with higher glucose content (76 mol%) and lesser glucosamine (16 mol%) content. For the extraction approach in the absence of reducing conditions, the main glycosidic linkages were (1→6)-Glc (17 mol%), (1→4) Glc (8 mol%), (1→3)-Glc (7 mol%), and (1→3,4)-Glc (32 mol%). The glycosidic linkages extracted under reducing conditions (R_1M.NaOH_RA) were mainly (1→3)-Glc (26 mol%), (1→4) Glc (18 mol%), (1→6)-Glc (15 mol%), and (1→3,6)-Glc (14 mol%), showing a structure less branched and with higher content of (1→3)-linked glucose.

The hypocholesterolemic potential of characterized fractions were evaluated using a simplified *in vitro* intestinal model where the ppt_Sn1M.NaOH and all the fractions obtained with the alkaline extraction with reducing agent (supernatant, precipitate, and residue) reduced cholesterol bioacessibility (from 0.35 mM to 0.20-0.34 mM of soluble cholesterol), whereas no significant effect was observed for the remaining fractions. The decrease was associated with the higher content of (1→3)-Glc and (1→4)-Glc linked in these fractions. For comparison, mixed linked β(1→3)/(1→4)-glucans from oat and barley, which are already recognized by EFSA as cholesterol reducing ingredients, were used as a positive control decreasing soluble cholesterol from 0.35 mM to 0.15-0.19 mM, highlighting the relevance of structures containing together β(1→3)-Glc and (1→4)-Glc linked for this activity. The strategy that enabled recovery the glucan structures with hypocholesterolemic potential was the alkaline extraction using reducing agent. The extraction strategy combined with detailed structural characterization provides essential insights for the valorization of mushroom by-products as hypocholestolemic ingredients.

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Grain Silo Dust to Leather Industry: Exploring a Sustainable Biomass Source

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The valorization of agro-industrial by-products is increasingly recognized as a key strategy to promote circularity and environmental sustainability. Although grain silo dust (GSD) has been widely investigated from occupational health and safety perspectives, its physicochemical characterization and potential for industrial upcycling remain largely unexplored, particularly regarding its application as a renewable feedstock for bio-based leather finishing systems. As a fine particulate by-product recovered from aspiration systems in cereal storage silos, GSD may represent a valuable source of starch-rich biomass for bio-based applications. The growing interest in starch-based materials for sustainable leather finishing highlights the need to identify alternative and sustainable starch-rich feedstocks. Hence, this study characterized the physicochemical and technological properties of ten independent batches of GSD to explore their potential as a sustainable feedstock for starch-based leather finishing formulations.

A comprehensive physicochemical characterization of the GSD batches was performed, including particle size distribution (sieving), proximate composition (AOAC methods), starch content (enzymatic assay), structural carbohydrates and lignin (NREL method), water solubility at different agitation times, Fourier transform infrared (FTIR) spectroscopy, rheological (viscosity), and thermal analysis by differential scanning calorimetry (DSC).

Particle size distribution analysis showed that GSD consisted predominantly of fine particles, although the relative abundance of each size fraction varied among batches. In nine out of ten batches, particles smaller than 150 µm accounted for 71–90% of the material, whereas in the remaining batch, they accounted for 55%. The low proportion of coarse particles indicates that the by-product may be readily incorporated into bio-based formulations without additional milling. Moreover, moisture content ranged from 12 to 15 g of water/100 g, indicating that only limited drying may be required to ensure material stability. Together, these characteristics reduce the need for energy-intensive pre-processing steps, thereby supporting the economic and environmental sustainability of GSD valorization as a renewable feedstock for leather finishing formulations. Chemical composition analysis confirmed that GSD is a starch-rich by-product, with starch contents exceeding 60 g/100 g in most batches. Structural carbohydrates also accounted for a substantial portion of the GSD, whereas free sugars, fat, and ash were consistently present at low levels. Finally, protein content remained close to 5 g/100 g in most batches. FTIR spectra further confirmed the presence of characteristic functional groups associated with these components. Technological characterization showed that water solubility remained low, ranging from 6–8%, 6–9%, and 6–12% after 5 min, 30 min, and 24 h of stirring, respectively. This behaviour is consistent with the presence of structural carbohydrates and lignin in the material, which may limit polymer swelling and water dispersion. However, the limited water solubility suggests that formulation optimization may be required for leather finishing applications. Additionally, thermal analysis demonstrated good thermal stability until 200 °C, whereas rheological measurements indicated relatively low viscosity values, which could facilitate formulation processing and application.

Overall, the results demonstrate that GSD is a promising starch-rich agro-industrial by-product with physicochemical characteristics supporting its potential valorization as a renewable feedstock for starch-based leather finishing formulations. Although its limited water solubility may require formulation optimization, the combination of high starch content, favourable particle size distribution, low drying requirements, and good thermal stability supports its potential use in leather finishing formulations and warrants further application-scale validation studies within a circular bioeconomy framework.

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Monitoring prickly pear ripening to optimize harvest timing and reduce postharvest losses: integrating digital tools, NIR spectroscopy and chemical characterisation

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Prickly pear (*Opuntia ficus-indica*) is a resilient fruit crop well adapted to Mediterranean environments and increasingly valued for its nutritional and functional properties. However, determining the optimal harvest stage remains challenging due to the rapid physicochemical changes occurring during fruit ripening, which directly affect quality, marketability and processing suitability.[1] Inaccurate harvest timing may therefore contribute to postharvest losses and food waste.

This work evaluated complementary non-destructive approaches for ripening monitoring in prickly pear fruits, aiming to support harvest optimisation and improve value chain efficiency. Fruits at two maturity stages (mid-ripe and ripe) were characterised over two consecutive seasons using a smartphone-based ripeness estimation application,[2] and portable near-infrared spectroscopy (NIRS).[3] Conventional quality parameters, including total soluble solids (TSS), moisture and firmness, were measured to validate maturity progression.

The smartphone application provides an objective and continuous ripeness score from RGB images, enabling rapid field assessment without specialised equipment.[2] In parallel, NIRS measurements (310–1100 nm) were used to predict internal quality attributes through chemometric models.[3] The NIRS prediction models achieved R^2 values of 0.78 for TSS, 0.67 for moisture and 0.79 for firmness, the latter with a prediction error of 2.7 N (10% relative error).

To complement maturity assessment, fruits were chemically characterised through the determination of TSS, organic acids, phenolic compounds, and betalains. Ripening was associated with a marked increase in TSS (13.7 to 16.0 °Brix), fructose (181.0 to 276.3 g kg^{-1} DW) and total betalains (0.233 to 0.419 mg g^{-1} DW), accompanied by a substantial decrease in titratable acidity (17.9 to 8.1 meq 100 g^{-1} DW) and malic acid (5.26 to 3.18 g kg^{-1} DW). HPLC analysis identified piscidic acid, eucomic acid, feruloyl glycosides, and isorhamnetin glycosides, providing additional insight into the biochemical changes occurring during fruit maturation.

The integration of digital imaging, portable spectroscopy and chemical characterisation offers a comprehensive framework for objective ripening assessment in prickly pear. These tools may contribute to more accurate harvest scheduling, reduced postharvest losses and improved utilisation of fruit quality potential, supporting more sustainable and efficient production systems.

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From Waste to Wellness: Fish By-Product Bioactives for Liver Health

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The valorization of fish by-products through microbial fermentation and lipid recovery represents a sustainable strategy for producing high-value bioactive ingredients with potential health-promoting properties[1]. In this study, post-biotics generated through the controlled fermentation of fish by-products with *Bacillus subtilis* and lipid fractions extracted from the same raw materials are evaluated for their impact on hepatic metabolism. Fish-derived biomass is subjected to controlled fermentation using selected microbial strains, generating a complex mixture of metabolites resulting from the biotransformation of proteins, lipids, and other organic constituents. The resulting post-biotic fractions are characterized by using chromatographic and mass spectrometry-based approaches to determine their metabolite composition, while the extracted lipid fractions are assessed for their biological activity.

The effects of both ingredients are investigated using hepatic cell models, focusing on key markers of lipid metabolism, lipid accumulation, oxidative stress, and metabolic regulation. Attention is given to pathways involved in fatty acid oxidation, lipogenesis, and cellular redox balance. In hepatic steatosis models, the lipid fractions extracted from fish by-products reduced intracellular lipid accumulation by approximately 30%, demonstrating a promising capacity to attenuate excessive fat deposition in liver cells. The integration of metabolite profiling with cellular bioactivity data provides valuable insights into the mechanisms through which fish-derived post-biotics and lipid fractions influence hepatic metabolic function.

These findings contribute to the development of innovative functional ingredients from marine by-products and support the exploitation of fish-derived bioactive compounds as sustainable solutions for applications in food, nutraceutical, and health-related sectors.

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From Pineapple Waste to Water Treatment: Developing Sustainable Tannin-Based Biocoagulants

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The increase in industrialization in the agri-food sector generates large amounts of food waste and starch-rich wastewater, especially from potato peel processing, which contains elevated levels of total suspended solids, turbidity, and chemical oxygen demand, posing risks to aquatic ecosystems when discharged[1]. Therefore, treatment is essential before this wastewater is discharged, and one procedure often used to treat wastewater before disposal is coagulation, which is known to achieve high removal efficiencies at low cost. Conventional coagulants, such as iron chloride and aluminum sulfate, are effective; however, they produce large amounts of sludge, operate within a limited pH range, and the compounds present in aluminum coagulants are linked to neurodegenerative diseases like Alzheimer's[2]. Consequently, there is a need for new eco-friendly alternatives to these conventional coagulants, and one such alternative is tannin-based coagulants derived from agricultural residues[2,3]. Hence, this work aims to evaluate tannin-based coagulants produced from residues of CAMPOTEC, a Portuguese fruit and vegetable producer organization, for the treatment of its potato-processing wastewater and to compare their performance with commercial Tanfloc and conventional coagulants, iron chloride, and aluminum sulfate. The wastewater was pretreated by centrifugation and characterized before coagulation-flocculation tests due to the high level of solid waste. Before the Jar Tests, 0.5 g of NaCl was added to facilitate particle aggregation and flocculation. After the wastewater is characterized, it shows an average turbidity of 70 NTU and a color of 140 Pt/co. Through a Jar Test using the previously mentioned coagulants, Tanfloc achieved the highest turbidity removal of 87% at 60 mg/L, outperforming both conventional coagulants: iron chloride at 68% at 30 mg/L and aluminum sulfate at 81% at 20 mg/L. The next step was the production of tannin-based coagulants using residues provided by CAMPOTEC. After a literature review, the selected residues were spinach and pineapple peel and crown. Initial assays to determine their phenolic content showed that the pineapple peel had higher phenolic concentrations, reaching 495 mg-GAE/L after extraction at 60 °C for 1 h. After selecting the residue, an experimental design in Minitab was used to determine the best solid-liquid extraction conditions using distilled water, varying the water-to-solid ratio, temperature, and extraction time. Achieving an extraction of 639 mg-GAE/L, with a ratio of 1:50 (solid: liquid) for 35 min at 90 °C. After selecting the best extraction result for the pineapple peel residue, the next steps will be to determine the tannin content present and use the extract to produce biocoagulants to treat CAMPOTEC's wastewater. These preliminary results demonstrate the potential of pineapple peel residues as a sustainable source of tannin-rich extracts for producing biocoagulants, contributing to both wastewater treatment and agro-industrial waste valorization.

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Production and Characterisation of Green Banana Flour (GBF) from an Autochthonous Variety of the Autonomous Region of Madeira: Bromatological Composition and Sensory Evaluation in Bread-Making and Pastry Formulations

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Banana (Musa sp.) is the most widely produced and consumed tropical fruit worldwide, playing a central role in the agricultural economy of the Autonomous Region of Madeira (RAM), with a total production of 25,688 tonnes in 2024 (DREM, 2025). In that same year, 24,500 tonnes were marketed, indicating that approximately 1,200 tonnes did not meet the criteria for size, ripeness, or visual conformity required for commercialization, resulting in post-harvest losses with significant economic impact. There is a loss of green banana production that does not reach the market because it fails to meet required quality standards. This by-product is diverted to composting, animal feed, on-farm consumption, etc. The strategy is to add value to this by-product by transforming it into a functional food. Within the framework of the circular economy and the valorisation of agri-food by-products, this study aimed to develop and characterize a process for obtaining green banana flour (GBF) from non-marketable fruits, with and without peel, evaluating its technological performance and sensory acceptability in bread-making and pastry applications.

The production process comprised the following sequential stages: raw material reception and selection, washing and separation of banana fingers, peeling and/or particle size reduction, oven-drying at controlled temperature, milling, and flour packaging. Bromatological characterization — including determination of moisture content, total lipids, available sugars, total starch, and total dietary fibre — was carried out by the University of Madeira (UMa) using validated official methods. The bromatological analysis was conducted by UMa; the content or ratio of amylose to amylopectin was not determined, although this is something intended for the future. Sensory evaluation was conducted by AgroSenLab through a trained panel and consumer trials, using formulations with partial and total substitution of wheat flour (WF) by GBF at ratios of 30%, 50%, and 100% (w/w).

Production yields were 32.1% for GBF with peel and 15.5% for GBF without peel. Bromatological analysis revealed moisture content below 10% (w/w), low concentrations of total lipids and available sugars, and high levels of resistant starch and total dietary fibre, conferring on GBF a nutritional profile consistent with that of functional ingredients suitable for low-glycaemic-index products. Sensory evaluation demonstrated overall good acceptance and positive purchase intention for the majority of the developed products, with distinction for breads and brioches formulated with peelless GBF at substitution levels of 50% and 100%, which achieved the highest hedonic scores.

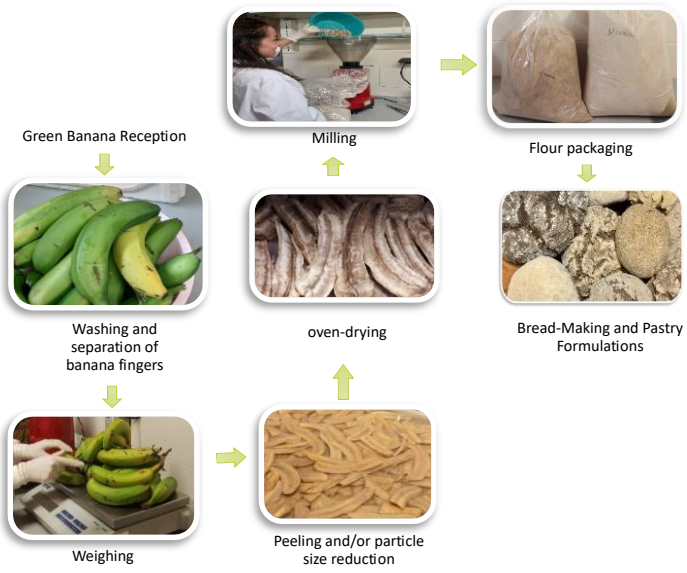


Figure 1 - Flowchart of the green banana flour (GBF) production process

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From Insect Exuviae to Chitosan: Characterization and Ultrasound-Assisted Demineralization of Black Soldier Fly Residues

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Insects have received increasing attention as a source of protein, lipids, and biopolymers due to their efficient resource utilization and ability to valorize organic side streams.1 Among the species currently produced at industrial scale, the black soldier fly (Hermetia illucens) is one of the most widely studied and commercially relevant.2 The expansion of black soldier fly production generates increasing quantities of by-products, including exuviae, which have been identified as a potential source of chitin. Compared with crustacean-derived chitin, however, data on the composition of insect exuviae, extraction performance, and product characterization remain limited.1

This study evaluated black soldier fly exuviae as a feedstock for chitin and chitosan production, with particular emphasis on raw material characterization and demineralization performance. Chitin was recovered through demineralization, deproteinization, and decolorization, followed by deacetylation to obtain chitosan. In addition, ultrasound-assisted demineralization was investigated as a potential approach to reduce acid concentration and processing time during the initial extraction step.

The exuviae contained 11.3% moisture and, on a dry matter basis, 16.4% ash, 13.9% lipids, and 28.9% chitin. Protein content was estimated at 43.8% based on nitrogen determination corrected for the chitin fraction.

A conventional demineralization treatment (1 N HCl, 30 °C, 1 h, S/L ratio 1:10) resulted in a demineralization yield of 69.3% and a residual ash content of 0.23%. To evaluate whether comparable mineral removal could be achieved under less severe conditions, the acid concentration and treatment time were reduced to 0.5 N HCl and 30 min. Under these conditions, demineralization without ultrasound resulted in a yield of 68.7% and a residual ash content of 0.60%, whereas ultrasound-assisted treatment resulted in a yield of 64.5% and a residual ash content of 0.41%. While the conventional treatment achieved the lowest residual ash content, the ultrasound-assisted process improved ash removal relative to the corresponding non-ultrasound treatment operated under identical conditions.

FTIR analysis showed the progressive removal of non-chitin components and the formation of characteristic chitin and chitosan bands throughout the extraction process (Figure 1).

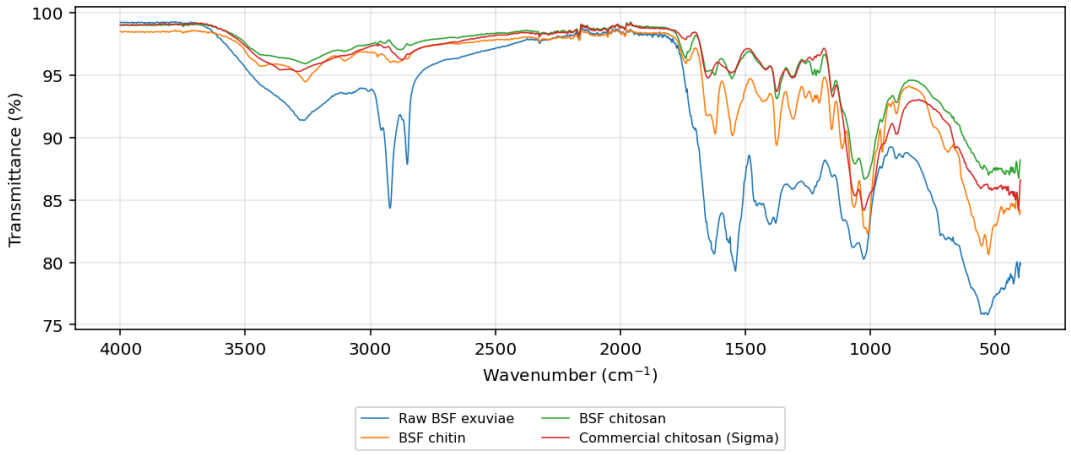


Figure 1 - FTIR comparison of processing steps and commercial chitosan. Spectra of raw black soldier fly (BSF) exuviae, extracted chitin (after demineralization, deproteinization, and decolourization using $CHCl_3$/EtOH/H_2O and H_2O_2), insect-derived chitosan, and commercial chitosan.

Chitosan was further characterized by determination of the degree of deacetylation (DDA) using a potentiometric titration method and compared with a commercial reference material.3 The produced chitosan exhibited a DDA of 84.3%, compared with 83.9% for the commercial sample. During potentiometric analysis, most of the produced chitosan dissolved, although a small residual insoluble fraction remained. This observation may indicate the presence of residual impurities or incompletely converted material and should be considered when interpreting the DDA results.

Despite the successful conversion of chitin to chitosan, the overall chitosan yield corresponded to only 1.9% of the initial dry biomass. Yield losses were particularly pronounced during the deacetylation step, indicating that process conditions require further optimization.

Overall, the processing sequence enabled the recovery of chitin from black soldier fly exuviae and its subsequent conversion into chitosan. However, several methodological and process-related limitations were identified. Chitin quantification remains affected by the limited standardization of analytical approaches for insect-derived materials, while protein estimates depend on the nitrogen-to-protein conversion factor applied. In addition, the low final chitosan yield and challenges during decolorization indicate that further optimization is required.

These findings provide compositional and processing data for black soldier fly exuviae and illustrate some of the analytical and technological constraints currently associated with insect-derived chitin production or its modification to chitosan.

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[O.5.15]

Impact of salinity conditions on the sensory properties of *Salicornia Ramosissima*

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Halophytes such as Salicornia ramosissima are emerging as sustainable alternatives to conventional salt due to their natural sodium chloride accumulation. They are also valued in gastronomy for their distinctive sensory characteristics, nutritional composition[1], and potential to enhance flavour. However, environmental conditions may affect their sensory quality and consumer acceptance. Soilless cultivation systems enable precise control of salinity and other cultivation parameters, improving water-use efficiency and product consistency. In collaboration with RiaFresh®, this study evaluated the effect of salinity on the sensory quality of three Salicornia ramosissima genotypes (SR1, SR2, SR3) cultivated under three salinity conditions (C1, C2 and C3), aiming to identify the cultivation conditions associated with the most favourable sensory profile.

Sensory evaluation was conducted by a panel of 11 professional chefs selected for their culinary expertise and ability to provide informed assessments of sensory quality. Overall acceptance was assessed using a nine-point hedonic liking scale, and a Just-About-Right (JAR) test was performed to evaluate the intensity of eight key sensory attributes. Crunchiness, saltiness, bitterness, citrus note, and juiciness were rated as below, at, or above the ideal intensity, whereas fibrousness, herbal character, and astringency were classified as at the ideal level, acceptable, or above the ideal level. Liking scores were assessed for differences using Analysis of Variance (ANOVA), while JAR responses were explored using Multiple Correspondence Analysis (MCA) in XLSTAT to identify the sensory attributes most strongly associated with product acceptance. Overall liking scores and JAR responses were related using penalty analysis.

No significant differences were found for liking between the processing conditions for SR1 ($p > 0.05$). In contrast, significant differences were observed for SR2, where plants cultivated at salinity C1 received higher liking scores than C2 ($p < 0.05$), while C3 did not differ significantly from either condition. For SR3, C2 achieved the highest liking scores and was rated significantly higher than the remaining conditions ($p < 0.05$).

The first two dimensions of MCA explained 62.05% of the total inertia and differentiated the sensory profiles according to genotype and salinity conditions (Figure 1). SR3 under salinity C2 was associated with several JAR attributes, suggesting a more balanced sensory profile. SR1 was closely associated with saltiness and citrus notes at JAR levels under the same condition, while SR2 under salinity C2 and C3 was associated with non-ideal sensory perceptions, including excessive bitterness and insufficient crunchiness and juiciness. These findings indicate that the effect of salinity on the sensory quality of the three genotypes differed under the same salinity condition. Other genotype/conditions associations were positioned more towards non-JAR responses, mainly involving excessive crunchiness, juiciness or saltiness, and insufficient citrus character.

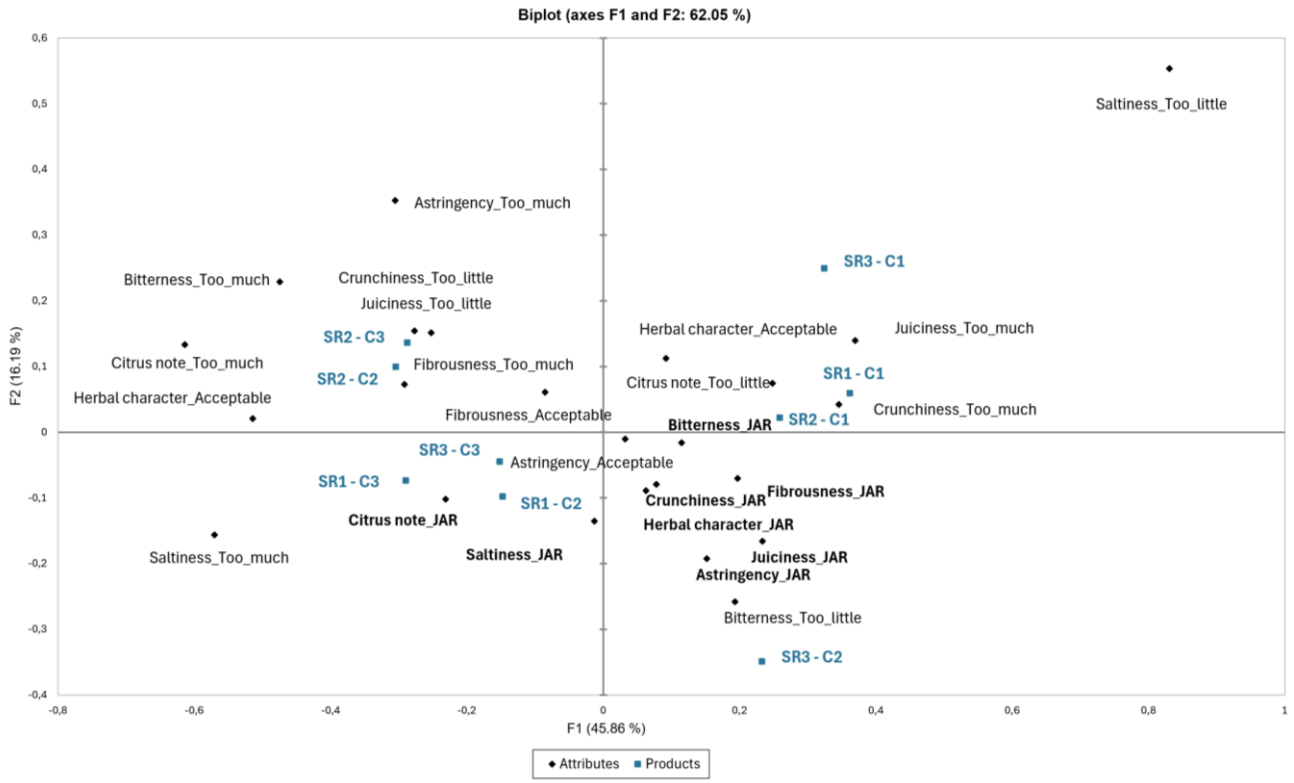


Figure 1 - MCA biplot shows the association between the three Salicornia ramosissima samples (SR1-SR3), each grown under a different salinity condition (C1-C3), and JAR sensory attributes. Blue squares represent samples and black squares represent attributes. The first two dimensions explain 62.05% of the inertia (F1: 45.86%; F2: 16.19%).

Penalty analysis combining all data further identified astringency as the sensory attribute with the greatest negative impact on overall liking, followed by herbal character, bitterness, juiciness, and fibrousness.

The results demonstrate that the sensory response to salinity appears to be genotype-dependent, with distinct Salicornia ramosissima genotypes exhibiting different sensory profiles and levels of acceptance, even within the same salinity condition. These findings highlight the importance of optimising cultivation conditions for each genotype to optimise sensory quality and support the use of Salicornia ramosissima as a sustainable gastronomic ingredient.

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[O.5.16]

Valorisation of PGI and PDO Fruits: Effect of Blanching and Antioxidants on the Physicochemical, Rheological and Sensory Stability of Fruit Pulps

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The valorisation of fruit surpluses and visually rejected fruits represents an important strategy to reduce food waste and promote sustainable food systems, particularly through the recovery of dietary fiber and bioactive compounds from fruit by-products[1,2]. Fruit pulps represent a promising strategy for extending shelf life while preserving the nutritional and functional properties of fruits, including dietary fibre and bioactive compounds. This study evaluated the influence of blanching and antioxidant incorporation on the physicochemical, rheological and sensory stability of fruit pulps during 25 days of storage. Fruit pulps were produced from surplus and visually rejected Fuji and Gala apples from the "Maçã de Alcobaça" Protected Geographical Indication (PGI) and Rocha pear from the "Pera Rocha do Oeste" Protected Designation of Origin (PDO). The pulps were subjected to three processing conditions: untreated pulp, blanched pulp (B), and blanched pulp with antioxidant addition (ascorbic and citric acids) (BA). Physicochemical analyses included pH, soluble solids content (°Brix), colour parameters (L*, a*, b*), viscosity and rheological behaviour. Sensory characterisation was performed using Quantitative Descriptive Analysis (QDA) with a trained sensory panel. Statistical analysis was carried out by ANOVA followed by Tukey's test ($p < 0.05$).

The processing treatments significantly affected pulp yield and quality attributes. Rocha pear exhibited the highest initial pulp yield (91.56 ± 0.00%), which decreased after blanching (85.67 ± 0.00%) and further declined following antioxidant incorporation (77.35 ± 3.60%). Similar trends were observed for Fuji apple, with pulp yields decreasing from 90.75 ± 0.01% in untreated samples to 81.67 ± 9.01% after blanching and antioxidant addition. In contrast, Gala apple showed greater stability, maintaining yields between 85.25 ± 1.66% and 88.68 ± 0.05% (Figure 1A).

Soluble solids remained relatively stable in apple pulps, ranging from 13.8 ± 0.5 to 14.5 ± 0.3 °Brix, whereas Rocha pear showed a significant reduction after blanching, decreasing from 16.1 ± 0.2 °Brix (PR) to 14.7 ± 0.3 °Brix (PRB). Antioxidant addition partially mitigated these losses (15.2 ± 0.4 °Brix) (Figure 1B). Similar effects of thermal processing on physicochemical properties and antioxidant stability have been previously reported for fruit pulps subjected to blanching and preservation treatments[3].

The incorporation of antioxidants significantly reduced pH values and improved physicochemical stability. Fuji apple pulp showed a decrease from 4.21 ± 0.03 (MF) to 3.78 ± 0.01 (MFBA), while Gala apple decreased from 3.95 ± 0.02 to 3.74 ± 0.02. Rocha pear presented higher pH values overall, ranging from 4.52 ± 0.03 to 4.28 ± 0.03 (Figure 1C).

Colour measurements highlighted the protective effect of antioxidants against enzymatic browning. Antioxidant-treated samples exhibited higher luminosity (L*) values and lower redness (a*) values compared with untreated pulps. Rocha pear pulp with antioxidant addition showed the highest luminosity (43.89 ± 0.11) (Figure 1D), while untreated Gala apple exhibited the greatest browning tendency (a* = 8.44 ± 0.11) (Figure 1 E). In Fuji apple, antioxidant incorporation increased luminosity from 30.62 ± 0.33 to 38.11 ± 0.10, indicating a marked improvement in colour preservation.

All fruit pulps exhibited non-Newtonian pseudoplastic behaviour ($n < 1$), characteristic of fruit-based dispersions. Antioxidant-treated samples showed greater structural stability, with the highest consistency index observed for Gala apple pulp with antioxidants ($k = 31741$ mPa·s^n; $n = 0.214$). Viscosity values ranged from 980 ± 85 mPa·s (PRB) to 1505 ± 130 mPa·s (MFBA) (Figures 1F, 1H and 1I), suggesting enhanced resistance to structural breakdown during storage.

Sensory characterisation by QDA revealed significant differences among treatments, particularly in colour uniformity, browning perception and texture-related attributes. Pulps subjected to blanching combined with antioxidant addition were characterised by greater visual attractiveness, lower browning intensity and improved texture homogeneity. These samples also achieved the highest scores for overall sensory quality, demonstrating that antioxidant incorporation effectively preserved desirable sensory attributes throughout storage. The sensory findings were consistent with the instrumental colour and rheological measurements, confirming the positive impact of the preservation strategy on product quality (Figures 1 J, 1L, and 1M).

Overall, the combination of blanching and antioxidant incorporation improved colour preservation, physicochemical stability, rheological behaviour and sensory quality of fruit pulps produced from surplus apples and pears. These findings demonstrate the feasibility of transforming non-commercial fruits into stable, value-added food ingredients, contributing to food waste reduction, sustainable processing practices and the promotion of circular economy approaches within the fruit sector.

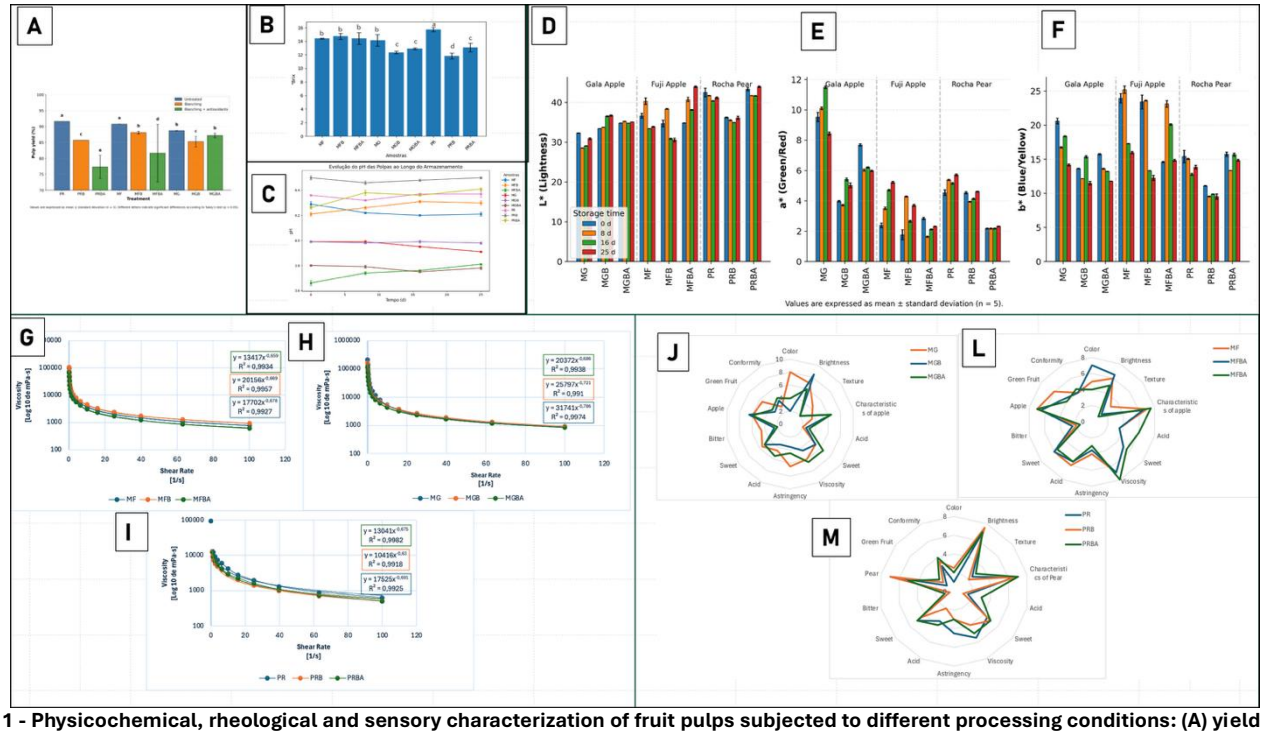


Figure 1 - Physicochemical, rheological and sensory characterization of fruit pulps subjected to different processing conditions: (A) yield (%); (B) soluble solids content (°Brix); (C) pH values during storage; (D) luminosity parameter (L*); (E) red-green coordinate (a*); (F) yellow-blue coordinate (b*); (G–I) rheological and viscosity profiles; and (J–M) sensory evaluation of fruit pulps.

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Unlocking Tyrosine from Sheep Whey Proteins: A Novel Pre-Treatment Strategy for Sustainable Bacterial Eumelanin Production

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Sheep whey is one of the most abundant by-products generated by the dairy industry in the Beira Baixa region of Portugal, where its management remains a significant environmental and economic challenge for small and medium-sized cheese producers. Despite being commonly regarded as a low-value residue, sheep whey contains substantial amounts of high-quality proteins that represent an underutilised source of amino acids for biotechnological applications[1,2].

This study proposes a novel valorisation pathway for sheep whey based on its use as a tyrosine-rich substrate for bacterial eumelanin production. Previous work by our research group demonstrated the ability of Pseudomonas putida ESACB 191 to synthesise a natural brown eumelanin pigment with antioxidant activity and remarkable functional stability, highlighting its potential for applications in food, cosmetic and biomaterial sectors[3]. However, pigment production is constrained by the limited bioavailability of tyrosine, which is predominantly incorporated
within stable whey protein structures.

To overcome this limitation, a thermal pre-treatment strategy was developed to maximise the release of free amino acids, particularly tyrosine, prior to microbial cultivation. Sheep whey samples were subjected to four treatment conditions at 100 °C for 15 and 30 min, with and without pH adjustment to the isoelectric point of whey proteins (pH 4.5) using hydrochloric acid. The combination of heat-induced denaturation and isoelectric precipitation was expected to promote protein destabilisation and enhance amino acid accessibility.

The chemical composition of both whey and the resulting hydrolysates was monitored using MilkoScan™ analysis, enabling substrate standardisation and the assessment of compositional changes throughout processing. Following centrifugation, total free amino acids were quantified using the ninhydrin assay, while residual soluble protein content was determined by the Biuret method. The combined evaluation of these parameters provided a robust assessment of protein disruption efficiency and amino acid release under the different processing conditions.

The optimal pre-treatment identified in this work will support a subsequent enzymatic hydrolysis stage using food-grade proteases to further enhance tyrosine availability. By integrating thermal denaturation and enzymatic hydrolysis, this research aims to convert sheep whey into a high-value biotechnological feedstock for microbial
eumelanin production.

To the authors' knowledge, this is among the first studies to investigate sheep whey specifically as a precursor source for bacterial eumelanin biosynthesis. The proposed approach contributes simultaneously to agro-industrial waste valorisation, natural pigment production and the development of circular bioeconomy solutions aligned with the objectives of the European Green Deal and sustainable resource management strategies[1,2,4].

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Validation of an In Situ Derivatization Method for GC-MS Assessment of TBBPA and EE2 Bioaccessibility in Mackerel after *In Vitro* Gastrointestinal Digestion

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Endocrine disruptors (EDs) such as tetrabromobisphenol A (TBBPA) and 17α-ethinylestradiol (EE2) are emerging contaminants that may accumulate in fish and pose potential health risks through dietary exposure 1. Upon ingestion of fish, a fraction of compounds is released from the food matrix during gastrointestinal digestion (GID), becoming available for absorption – bioaccessibility. Therefore, assessing contaminants bioaccessibility is crucial. However, this can be highly complex, as the bioaccessible fraction resulting from digestion contains digestive enzymes, bile salts, broken-down food components and digesta fractions and analytical methods for determining TBBPA and EE2 in complex digesta matrices remain limited. This study optimised and validated an in situ derivatization procedure following acetonitrile-based extraction and before GC-MS/MS analysis, for the simultaneous determination of bioaccessible TBBPA and EE2 generated by the INFOGEST 2 static in vitro GID of mackerel. The method was subsequently applied to determine the bioaccessibility of both compounds at different spiked levels, providing, to our knowledge, the first assessment of the bioaccessibility of TBBPA and EE2 in fish following in vitro GID.

Mackerel was steamed for 20 minutes (20 cm above boiling water) and homogenised with a stainless-steel blender. GID was simulated following the in vitro static INFOGEST 2.0 digestion protocol. The resulting bioaccessible fraction (supernatant) was extracted using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure. Thus, the bioaccessible fraction was extracted with acetonitrile, followed by the addition of NaCl and $MgSO_4$. After vigorous mixing and centrifugation, the supernatant was filtered and cleaned using and using Supel™ QuE Z-Sep Bulk. After vortexing and centrifugation, the resulting extract was used to optimise derivatization. The combined effects of three independent variables (volumes of acetic anhydride, chloroform and pyridine) were studied using a 23 full-factorial Central Composite Design (CCD). The conditions for in situ derivatization and extraction were optimised using response surface methodology to maximize the GC–MS/MS analytical response. From experimental results, CCD indicated that optimal conditions for the studied variables were the lower assessed volume of chloroform, the higher assessed volume of pyridine and 175 µL of acetic anhydride.

The analytical performance of the method was evaluated. Excellent linearity was achieved for matrix-matched calibration from 2.5 – 50 µg L^{-1} for TBBPA and 0.5 – 10 µg L^{-1} for EE2, with correlation coefficients above 0.995. Good recoveries were achieved (82 – 92% and 84 – 113% of TBBPA and EE2, respectively). Limits of quantification were set at 2.5 µg L^{-1} and 0.5 µg L^{-1} for TBBPA and EE2, respectively.

The validated method was successfully applied to digestates obtained from mackerel by fortifying before in vitro GID at three different levels: 0.40, 0.80, 1.00 µg g^{-1} mackerel of TBBPA and 0.08, 0.16, 0.20 µg g^{-1} mackerel of EE2. Mean bioaccessibility values (mean ± SD, n = 2 independent digestions) were of 21.83 ± 4.07%, 22.33 ± 4.63%, and 22.43 ± 0.88% for TBBPA, and 13.38 ± 7.04%, 13.53 ± 2.93%, and 15.95 ± 1.27% for EE2, respectively. Comparable values of mean bioaccessibility across the different fortification levels support the applicability of the method, suggesting no marked concentration-dependent effect, although higher variability was observed for EE2 at the lowest level. TBBPA was consistently more bioaccessible than EE2. Although both compounds likely establish hydrophobic interactions with mackerel matrix components, namely protein and lipids, their different ionisation behaviour may have influenced their release during digestion the most. TBBPA becomes ionised at intestinal pH, increasing its aqueous solubility which may facilitate its transfer to the bioaccessible fraction. Contrarily, EE2 remains predominantly non-ionised throughout the digestion, potentially favouring interactions with the hydrophobic components of mackerel and its retention in the residual matrix, limiting its bioaccessibility. Although differences in molecular size may affect diffusion and partitioning, molecular weight is unlikely to explain this result (543.9 vs 296.4 g mol^{-1}, TBBPA vs EE2 respectively). Potential differences in chemical stability also cannot be excluded, although preferential transformation of EE2 could not be confirmed as only the parent compounds were assessed.

Overall, the optimisation and validation of the derivatization procedure, together with its application to mackerel digestates, support the suitability of the method for assessing TBBPA and EE2 bioaccessibility in the digestate of complex food matrices.

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Acorn Flour as a Sustainable Excipient for Food and Pharmaceutical Applications

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Acorn flour (AF), derived from Quercus rotundifolia, represents a sustainable and renewable natural product with substantial potential as a pharmaceutical excipient due to its high starch content, biodegradability, abun-dance, and multifunctional technical properties. Acorns, the fruits of Quercus species, contain approximately 40–60% starch, along with proteins, lipids, minerals, and bioactive compounds, making them excellent renewa-ble raw materials for both food and therapeutic applications. In addition to their ecological significance in sup-porting forest ecosystems, acorns have been traditionally used for food and medicine. The substitution of plant-derived excipients with synthetic alternatives is increasingly being recognized as an essential strategy to enhance the sustainability of pharmaceutical formulations. Furthermore, acorn flour has emerged as a cost-effective and gluten-free alternative to conventional excipients, supporting the development of environmental-ly friendly pharmaceutical products. The purpose of this study was to determine if AF is a suitable natural ex-cipient for the creation of directly compressed tablets by analyzing its physicochemical properties and the way it affects tablet performance, with a focus on drug dissolution and disintegration.

Particle size distribution analysis and differential scanning calorimetry (DSC) were used to evaluate the thermal behavior and physical properties of AF. Additional physicochemical characterization included drying loss, bulk and tapped density measurements, and compression behavior to assess its suitability for direct compression. Formulations that included AF, hydroxypropyl methylcellulose (HPMC), microcrystalline cellulose (MCC), mag-nesium stearate, and stevia were used as direct compress tablets. To optimize the formulation, a Design of Ex-periments (DoE) method was used to evaluate how different the excipient proportions affected tablet perfor-mance. Studies on hardness, disintegration, and dissolution were used to characterize the optimized formula-tions.

DSC study indicated that starch is the primary component responsible for the thermal transitions of AF, with the observed endothermic events in agreement with values reported for starch-rich botanical materials. The physicochemical characterization showed that AF exhibited a particle size distribution of d90 = 413 µm, d50 = 54 µm, and d10 = 13 µm, a drying loss of 5.67% (w/w), and bulk and tapped densities of 0.377 and 0.589 g/mL, respectively, indicating favorable compaction properties. Compression studies further demonstrated that AF exhibited compression behavior comparable to lactose monohydrate under loads up to 10 kN, approaching the performance of microcrystalline cellulose.AF exhibited excellent functionality during tablet manufacturing. Dis-solution trials showed that AF's swelling and water-wicking properties significantly increased tablet hydration and enhanced disintegration. Tablets containing AF achieved an approximately tenfold increase in drug disso-lution after 1 h compared with placebo tablets, indicating a considerable improvement in drug release perfor-mance while maintaining satisfactory tablet hardness. The potential of AF as a multifunctional natural disinte-grant was demonstrated by the DoE's successful identification of optimized formulations that ensured rapid disintegration with efficient dissolution.

These initial results show that acorn flour is a promising sustainable excipient for tablet formulations involving direct compression. Besides replacing conventional synthetic excipients, AF significantly improves tablet dissolution and disintegration, while displaying advantageous compression and physicochemical properties appropriate for pharmaceutical manufacturing. AF is a promising multifunctional excipient for sustainable pharmaceutical development due to its renewable origin, high starch content, good compactability, and improved tablet performance. Ongoing research focuses on optimizing AF-based formulations and evaluating their potential in vivo antidiabetic efficacy contributing to the development of sustainable pharmaceutical products based on renewable plant resources.

Keywords: Acorn flour; Sustainable excipient; Direct compression; Natural polymer; Drug dissolution; Tablet disintegration; Antidiabetic formulation

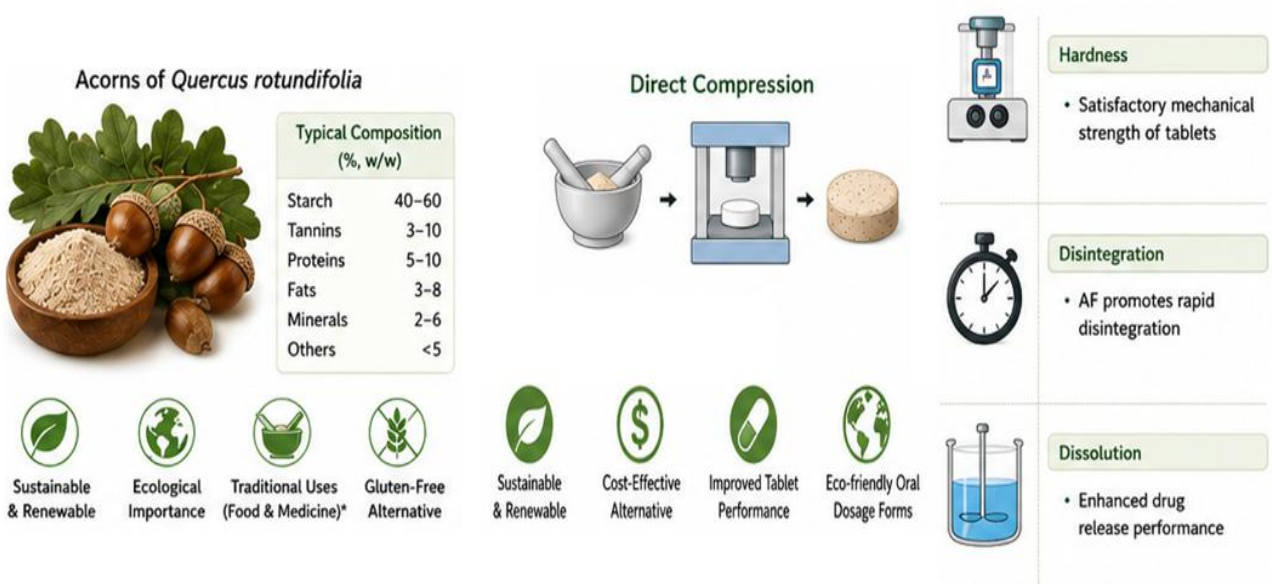


Figure 1. Composition and functional benefits of *Quercus rotundifolia* acorn flour as a pharmaceutical excipient.

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Unlocking the Potential of Sugar Refinery Molasses

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Sugar refinery molasses is a major agro-industrial by-product of sugar manufacturing, with an estimated global production exceeding 50 million tonnes annually. Molasses is still mainly used for low value applications, such as livestock and ethanol or biogas production via microbial fermentation. However, the high content of carbohydrates, minerals, and phenolic compounds suggests that molasses has strong potential as a valuable food ingredient. In this context, to better understand the opportunities and challenges associated with the valorisation of this by-product, this study aimed to characterise the composition and physicochemical properties of the high molecular weight fraction of sugar refinery molasses.

Molasses samples collected under different industrial storage conditions were characterized for their mono- and oligosaccharide profiles by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The polysaccharide composition was determined by accounting for the material retained in 12 kDa molecular weight cut-off membrane. The high molecular weight material was subsequently fractionated by C18 solid-phase extraction (SPE) at pH 6, allowing to obtain a non-retained hydrophilic fraction and a pH 6 retained hydrophobic fraction, which was eluted with methanol.

The pH of the non-retained hydrophilic fraction was adjusted to pH 3 and eluted again through the C18 cartridge. The pH 3 nonretained hydrophilic fraction was recovered and pH 3 retained hydrophobic fraction was eluted with methanol. The carbohydrate composition was determined by alditol acetate analysis using gas chromatography with flame ionisation detection (GC-FID). The glycosidic linkage composition was determined by methylation analysis of partially methylated alditol acetates using gas chromatography coupled with mass spectrometry (GC-MS).

The sugar refining molasses were mostly composed of 69.5 % of sucrose, 4.7 % glucose, and 1.4 % fructose. The polymeric material represented 0.99 % of sugar refining molasses. The pH 6 retained hydrophobic fraction accounted for 53 % of the sugar refining molasses high molecular weight material, mainly composed of 32.3 % glucose. The majority of glucosyl residues occurred as (1→4)-linked (28 mol %) and (1→4,6)-linked (23 mol %), suggesting the presence of small structures of amylopectin, and (1→6)-linked (23 %) suggesting the presence of microbial exo-polysaccharides, such as dextrans, possibly originated from lactic acid bacteria. The pH 3 retained hydrophobic fraction accounted for 11 % of the sugar refining molasses high molecular weight material, composed mainly by 12.1 % glucose, 4.7 % galactose and 4.7 % arabinose. The galactosyl residues occurred as (1→3,6)-linked (10 mol %) and arabinosyl residues appeared terminally linked (9.3 mol %) in a furanosidic form, characteristic of type II arabinogalactans. The pH3 non-retained hydrophilic fraction accounted for 32 % of the sugar refining molasses high molecular

weight material, composed mainly by 6.7 % glucose, 5.1 % galactose and 2.5 % arabinose. The glycosidic linkage pattern was also found to be characteristic of amylose, dextrans, and type II arabinogalactans.

These results demonstrate that besides sucrose, glucose and fructose, sugar refinery molasses contain amylopectin, dextrans, and arabinogalactans with hydrophobic and hydrophilic features. These findings yield new potentialities of sugar refinery molasses as functional ingredient of industrial relevance.

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Production of Pleurotus ostreatus on different agricultural by-product substrates from the RAM, yield and respective sensory evaluation

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Mushroom production in the Autonomous Region of Madeira (RAM) has been growing due to increasing consumer demand. In mainland Portugal, the fresh mushroom market has shown sustained growth, with an average annual growth rate of around 20% in recent years. In Madeira, there is a culture of mushroom consumption; however, the production of cultivated mushrooms remains incipient and small-scale, without its own representation in regional statistics, owing to the high production and substrate costs. Even so, there is a growing consumption trend, which motivated this trial using substrates derived from agricultural crops with significant regional relevance, following a rationale of waste valorization and production cost reduction. Besides their nutritional interest, mushrooms also have biotechnological and pharmaceutical potential.

This study was conducted within a collaboration between the University of Madeira and the Agri-Food Innovation Division (DIA) of the Regional Directorate of Agriculture (DRA), with the aim of evaluating the suitability of different regional agricultural by-products as substrates to produce Pleurotus ostreatus, promoting the valorization of agricultural by-products and the principles of the circular economy. Four substrates were tested, individually and as an equal-proportion mixture (Mix): apple pomace (BM), banana straw (PB), sugarcane bagasse (BC) and grape pomace (BU). Preparation involved cutting, shredding, moistening and pasteurising the residues, followed by inoculation with spawn, incubation in the dark (20–22 ºC, 80–90% humidity, 20 to 30 days) and fruiting (15–24 ºC, 85% relative humidity, 12-h photoperiod). Yield was assessed (kg fresh mushroom/kg substrate × 100), and a sensory evaluation was carried out by the AgroSenseLab Tasting Panel of the DRA, covering hedonic acceptance and purchase intention.

Apple pomace alone proved unviable due to contamination by Penicillium sp. and Rhizopus stolonifer but proved viable when incorporated into the Mix. The BC and BU substrates yielded only two flushes, whereas PB and Mix yielded three. The Mix showed the highest overall yield, followed by banana straw. In terms of sensory evaluation, grilled Mix mushrooms were the most appreciated, but the least preferred when consumed raw; BU and BC, grilled, achieved good hedonic acceptance just after the Mix, and banana straw mushrooms, raw, also achieved good results.

It is concluded that P. ostreatus production is viable on all four substrates evaluated, with the Mix being the most productive and profitable, followed by banana straw, demonstrating the potential for valorizing regional agricultural by-products in mushroom production.



Fig. 1 Fruiting of *Pleurotus ostreatus* on different substrates: apple pomace, banana straw, sugarcane bagasse, grape pomace, and mixed substrate.



Fig.2 Production of *Pleurotus ostreatus* on banana straw and fruiting with aromatic plants (fennel).



Fig.3 Comparison of the fruiting of *Pleurotus ostreatus* on banana straw and sugarcane bagasse.

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Optimizing acidogenic fermentation of lipid-rich food waste for value-added bioproducts

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The rapid accumulation of lipid-rich residues from frying operations has intensified the need for circular bioprocesses capable of converting these streams into high-value bioproducts. Spent frying oil (SFO) is particularly challenging due to the presence of toxic and recalcitrant compounds, which hinder conventional treatment and disposal. As a result, developing effective valorization strategies for SFO has become increasingly relevant. Acidogenic fermentation (AF) offers a promising route for transforming lipid-rich residues into short-chain organic acids (SCOAs) 1, versatile chemical platforms with potential for different applications, including the production of polyhydroxyalkanoates (PHA), a family of biodegradable polyesters. This work aimed to optimize AF conditions using SFO as the substrate and an uncharacterized mixed microbial population as acidogenic inoculum. Batch flask assays were conducted under mesophilic conditions at 30 oC across different feed-to-microorganism ratios (F/M) of 1:1, 1:2, and 2:1 g Chemical oxygen demand (COD) / g COD and three pretreatment strategies: CaCO3 buffering, partial substrate hydrolysis, and ultrasound treatment of the inoculum. Fermentation performance over ca. 30 days was monitored through pH measurement, High-performance liquid chromatography (HPLC) quantification of SCOAs, and Fourier-transform infrared spectroscopy (FT-IR) characterization of substrate and fermentation samples. Results for F/M of 1:1 showed that CaCO3 buffering reduced the maximum SCOAs concentration but broadened the acid-derived products profile, whereas partial hydrolysis and ultrasound pretreatments enhanced overall acids production, particularly acetic acid. Based on these outcomes, CaCO3 buffering was selected for follow-up experiments across F/M conditions of 1:2 and 2:1. Overall, these findings demonstrate that pretreatment strategies must be tuned to lipid-rich feedstocks to maximize SCOAs yield and support downstream PHA production. Ongoing work focuses on optimizing continuous-operation and scale-up the process in bioreactor, reinforcing AF as a circular and scalable approach for converting lipid-rich residues into high-value biochemical platforms.

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Assessment of the antioxidant and phenolic potential of canteen fruit peel residues for cosmetic and nutraceutical applications

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Converting agri-food by-products into value-added ingredients or products supports sustainability and the circular economy. On the Lusófona University campus in Lisbon, three canteens serve a community of approximately 13,000 people and generate around 2000 kg of fruit peel by-products each year, which can be reused and recycled [1].

Research was conducted to explore the valorisation of fruit peels as innovative ingredients for the food, pharmaceutical, and cosmetic sectors. This approach enables the recovery of organic by-products generated in canteens and their conversion into value-added raw materials, including extracts rich in bioactive compounds, antioxidants, and essential oils. Mango, orange, and pineapple peels generated in canteens were collected, separated by fruit type and processed into flours by oven-drying at 50°C for 24 hours, followed by grinding and sieving (0.6 mm sieve).

Hydroalcoholic extracts were produced with the flours by Soxhlet extraction (6 complete cycles) using a sample-to-solvent ratio of 1:10 (m/v) and ethanol/H_2O (70:30, v/v) solvent, followed by concentration under reduced pressure using a rotary evaporator.

Concentrated hydroalcoholic extracts were assessed for antioxidant activity (DPPH and FRAP spectrophotometric assays)[2], and their profiles of free sugars, organic acids and phenolic compounds were determined (HPLC-RI-PDA)[3]. Total phenolic and total flavonoid compounds were calculated by summing the individual phenolic and flavonoid compounds quantified by HPLC, respectively.

Statistical analysis by one-way ANOVA followed by Tukey's HSD test ($n = 3$ and $p < 0.05$) was performed with STATISTICA software.

The annual amounts of fresh by-products generated in canteens were 1,448, 266, and 102 kg/year for orange, pineapple, and mango, respectively. Following extraction, 90.4, 8.26, and 3.4 kg/year of concentrated dry extract were obtained, corresponding to extraction yields of 6.24%, 3.11%, and 3.33%, respectively.

The results showed no statistically significant differences in total sugar content among the three fruit peel flour extracts (167.2 ± 4.2 mg/g extract).

The mango peel flour extract stood out from the other extracts under study due to its higher antioxidant capacity (43.4±0.2 µmol TEAC/g extract and 64.6±7.0 µmol $FeSO_4$/g extract), and higher total levels of phenolic compounds (24.2±0.2 mg/g extract), flavonoids (13.9±0.1 mg/g extract) and organic acids (373.6±13.4 mg/g extract). Mango peel flour extract showed the highest content of sucrose, as well as lactic, formic, malic, caffeic, coumaric, ferulic, and ellagic acids, and rutin, apigenin, and myricetin. Orange peel flour extract had the highest content of naringin, procyanidin, and sinapic acid and pineapple had the highest content of kaempferol.

In conclusion, the mango extract demonstrated high antioxidant capacity, supporting its potential for use in natural cosmetic, nutraceutical, and pharmaceutical formulations.

The residues remaining after extraction were composted and subsequently applied to the campus's organic vegetable gardens (Figure 1), supporting circular economy and zero-waste practices.

The work promotes the UN Sustainable Development Goals for 2030: SDG 2 - Zero Hunger, SDG 9 - Industry, Innovation and Infrastructure and SDG 12 - Responsible Consumption and Production.



(A) (B)

Figure 1 - Composting of waste left over from the extraction processes (A) and application of the resulting organic compost in the University's vegetable gardens (B).

Keywords: mango, orange, pineapple, peel residues, extracts, antioxidant capacity, phenolic compounds, free sugars, organic acids
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Snacks Effect of Freeze-Drying and Hot-Air Drying on the Nutritional and Chemical Composition of Off-Grade *Vaccinium ashei* Pomace

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Blueberry (Vaccinium ashei) pomace, the main by-product generated during juice production[1], remains a valuable source of nutrients and bioactive compounds despite being derived from off-grade fruit[2]. Proper stabilization is essential to preserve its quality and support its valorization as a functional food ingredient. Therefore, this study evaluated the effect of freeze-drying and hot-air drying on the nutritional and chemical composition of off-grade blueberry pomace.

Off-grade V. ashei berries supplied by GreenFactor (Braga, Portugal) were processed to obtain blueberry pomace, which was subsequently stabilized by either freeze-drying (BPL) or hot-air drying (BPD). Instrumental color (CIELAB: L*, a* and b*) was evaluated[3], followed by the determination of proximate composition (protein, fat and ash) according to AOAC official methods. Free sugars and organic acids were analyzed by HPLC coupled to refractive index (HPLC-RI) and diode-array (HPLC-DAD) detectors, respectively. The fatty acid profile was determined by gas chromatography with flame ionization detection (GC-FID) following derivatization to fatty acid methyl esters (FAME). All analyses were performed in duplicate.

Freeze-dried pomace exhibited slightly higher redness (a*=14±1) than hot-air dried pomace (a*=11.9±0.4), whereas lightness (L*= 20.0±0.5) and yellowness (b* = 4.7±0.1 and 4.0±0.1, respectively) were similar between treatments. On a dry weight basis, protein contents were 3.4±0.1% and 3.6±0.1% in BPL and BPD, respectively, while fat content was approximately 2.0 g/100 g in both samples and ash contents were 0.3991±0.0001 and 0.3989±0.00003 g/100 g, respectively. Fructose and glucose were the predominant free sugars, glucose contents were 9.00 g/100g in both the samples while fructose was 8.5±0.1 g/100 g and 8.0±0.1 g/100 g in BPL and BPD, respectively. Malic acid was the predominant organic acid, with total organic acid contents of 3.9±0.6 and 4.7±0.2 g/100 g, respectively.

Linoleic (C18:2n-6) and oleic (C18:1n-9) acids were the predominant fatty acids, with polyunsaturated fatty acids accounting for approximately 49.2±0.4% and 49.2±0.3% in both samples, respectively.

Overall, freeze-drying and hot-air drying resulted in broadly similar nutritional and chemical compositions, with only minor differences in color. These findings indicate that both drying methods are suitable for stabilizing blueberry pomace prior to its valorization as a value-added functional food ingredient, supporting the sustainable use of off-grade blueberries within a circular bioeconomy approach.

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Valorisation of Leek (*Allium porrum L.*) By-products through Drying Technologies: Impact on Phenolic Compounds and Antioxidant Capacity of Different Plant Fractions

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Leek (Allium porrum L.) is a widely consumed vegetable recognised for its nutritional value and richness in vitamins, minerals, phenolic compounds, polysaccharides and sulphur-containing compounds, which are responsible for its characteristic aroma and biological properties, making it a food with potential health benefits[1,2,3]. However, a substantial proportion of leek production, particularly green leaves and vegetables that do not meet commercial quality standards (e.g., inadequate calibre or visual defects), is discarded despite retaining significant nutritional and functional value. The valorisation of these horticultural by-products represents a sustainable strategy to reduce food waste, promote circular bioeconomy practices, and generate value-added food ingredients with extended shelf life.

This study aimed to evaluate the antioxidant capacity of the upper part (green leaves) (Figure 1) and the lower part (stem and roots) of leek (Figure 2), to demonstrate the potential for valorisation and full utilisation of this vegetable. Furthermore, the effect of different drying technologies on the preservation of bioactive compounds and antioxidant capacity was assessed to support the sustainable utilisation of the entire leek biomass.



Figure 1- Leek Upper Part (UP).



Figure 2- Leek Lower Part (LP).

The effects of hot-air drying (50, 60, and 70 °C) and freeze-drying on the total phenolic content (TPC) and antioxidant capacity of leek were evaluated using the DPPH and ABTS assays, considering the upper part (UP) and the lower part (LP) separately. Statistical analysis included a two-way analysis of variance (ANOVA) for each parameter, followed by multiple comparisons among treatments using Tukey's or Games–Howell post hoc tests, and comparisons between the UP and LP within each treatment using Student's t-test or Welch's t-test.

Significant effects of treatment, plant fraction and their interaction were observed for TPC, DPPH and ABTS ($p<0.001$), demonstrating that the effectiveness of each preservation method depends on the leek fraction analysed. Fresh samples presented the highest antioxidant values. TPC ranged from 2.135 ± 0.04 mg GAE g^{-1} DW in the fresh LP to 0.239 ± 0.02 mg GAE g^{-1} DW in the freeze-dried UP (Figure 3). Among the preservation methods, hot-air drying at 60 °C yielded the highest retention of phenolic compounds (0.738 ± 0.00 mg GAE g^{-1} DW in the UP and 0.322 ± 0.02 mg GAE g^{-1} DW in the LP). Likewise, drying at 60 °C resulted in the highest antioxidant activity, with DPPH values of 0.248 ± 0.01 and 0.206 ± 0.01 mg Trolox equivalents g^{-1} DW, and ABTS values of 0.813 ± 0.03 and 0.518 ± 0.03 mg Trolox equivalents g^{-1} DW, for the UP and LP, respectively (Figure 4 and Figure 5).

For TPC, significant differences were observed among all treatments in the UP, whereas in the LP, only the fresh samples exhibited significantly higher values than the remaining treatments. Hot-air drying at 60 °C consistently preserved higher antioxidant capacity than drying at 50 °C or freeze-drying, as demonstrated by both DPPH and ABTS assays. Moreover, significant differences between the UP and LP were found for all evaluated parameters, highlighting the heterogeneous distribution of bioactive compounds within the plant and confirming that the effectiveness of the preservation method is strongly dependent on the leek fraction.

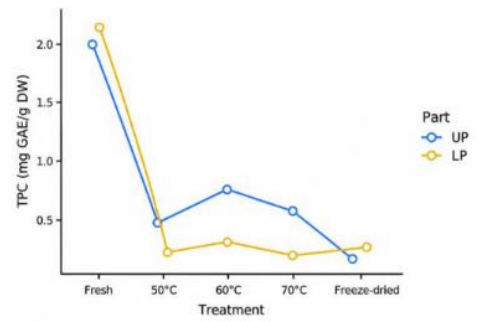


Figure 3- Total phenolic content (TPC) in UP and LP samples subjected to different treatments.

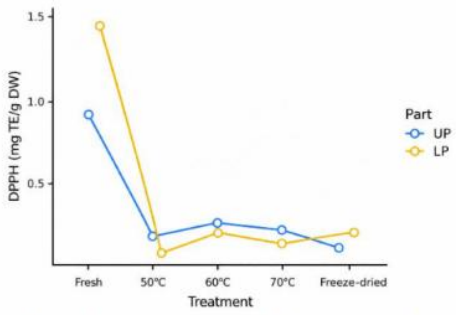


Figure 4- DPPH antioxidant activity in UP and LP samples subjected to different treatments.

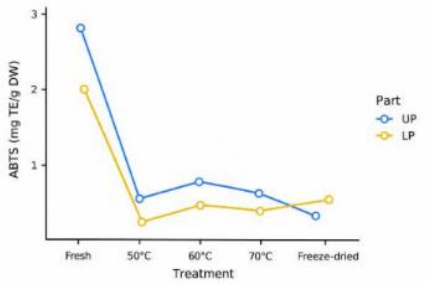


Figure 5- ABTS antioxidant activity in UP and LP samples subjected to different treatments.

Overall, the results demonstrate that hot-air drying at 60 °C is the most effective preservation method for maintaining the antioxidant properties of leek while extending its shelf life. These findings highlight the potential for sustainable valorisation of underutilised leek by-products and non-marketable vegetables, contributing to food waste reduction and supporting the development of functional food ingredients within a circular and sustainable agri-food system.

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Beyond phenolic extraction: integrated utilization of onion peel fractions for the development and nutritional evaluation of functional crackers

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The valorization of agro-industrial by-products has primarily focused on the recovery of high-value bioactive compounds, while comparatively less attention has been given to strategies that integrate both the recovered extract and the remaining biomass into functional food systems. Onion peel represents a promising raw material for this purpose due to its abundance of quercetin glycosides and dietary fiber. Considering the complementary roles of phenolic compounds and dietary fiber in modulating antioxidant availability, gut microbiota activity, and starch digestibility, the integrated utilization of both fractions represents a promising strategy for developing nutritionally enhanced cereal-based foods. Therefore, this study proposed an integrated approach to valorize onion peel by combining an extract rich in quercetin and its corresponding fiber extraction residue in the development of clean-label crackers.

A phenolic-rich extract was obtained through previously optimized microwave-assisted extraction (MAE) and characterized by UHPLC-HRMS, confirming quercetin glycosides as the predominant phenolic constituents. The extract was incorporated at 0.5% (w/w), while the corresponding extraction residue was used as a natural fiber source. Three formulations were produced: a control (C1) and two enriched crackers containing the same extract concentration and increasing levels of onion peel residue (C2 and C3). Nutritional composition, water activity, phenolic profile, and antioxidant activity were evaluated. Static in vitro gastrointestinal digestion (INFOGEST) was performed to assess phenolic bioaccessibility, followed by in vitro fermentation of the non-bioaccessible fraction to investigate its prebiotic potential. Starch digestibility and the in vitro glycemic index (GI) were also determined.

Increasing residue incorporation raised dietary fiber from 8.3% in C1 to 9.8% and 13.1% in C2 and C3, respectively. Although both enriched formulations contained the same amount of phenolic extract, total apparent phenolic bioaccessibility increased from 2.9% in C2 to 17.0% in C3. The bioaccessible fractions of the enriched crackers also showed greater antioxidant activity than the control, particularly in the DPPH assay, with EC50 values decreasing from 7.94 mg cracker/mL in C1 to 2.48 and 1.77 mg cracker/mL in C2 and C3, respectively. Increasing enrichment was further associated with a marked decrease in the estimated in vitro GI, from 94.4% in C1 to 75.7% and 69.0% in C2 and C3, while the non-bioaccessible fractions supported the growth of the tested probiotic microorganisms during in vitro fermentation. Overall, the combined use of the phenolic extract and fiber-rich extraction residue improved multiple nutritional and gastrointestinal functionality-related properties of the crackers, supporting an integrated strategy for the valorization of onion peel in clean-label cereal based foods.

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Sensory acceptability of porridge with and without flavouring made from blends of extruded flour from the peel and albedo of passion fruit (*Passiflora edulis f. flavicarpa Degener*) and rice (*Oryza sativa L.*)

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Extrusion is a thermomechanical process in which food materials containing starch or protein are subjected to a combination of moisture, pressure, temperature, and mechanical shear, resulting in molecular and physicochemical transformations such as partial or complete disruption of the semicrystalline structure, molecular fragmentation of starch polymers, and protein denaturation, thereby improving digestibility. The process includes proper ingredient preparation, conditioning to an adequate moisture content, and feeding the raw material into the equipment, where it is driven by the screw toward the die. As the product passes through the different extrusion zones, namely the feeding, transition, and high-pressure zones, mechanical friction gradually increases due to changes in screw geometry and die opening. Consequently, temperature and pressure also increase, leading to product cooking. The present study aimed to sensorially evaluate three porridge formulations with and without flavouring based on extruded flour blends of passion fruit peel and albedo with rice. A central composite experimental design was used to reduce the number of combinations and optimize data collection and analysis. Polished rice grains were milled in a disc mill using a 1 mm sieve, yielding white rice flour. After sanitation, the passion fruit was cut into four parts, the pulp was removed, and the peel and albedo were dried in an oven at 70°C for 4 h. After drying, the peels and albedo were weighed, packaged in plastic bags, and milled. The dried passion fruit peel and albedo samples were first ground in a hammer mill with a 1 mm sieve, then in a disc mill with a 2 mm opening, and subsequently in a Perten mill with a 0.8 mm sieve, yielding passion fruit peel and albedo flour. The pre-cooked mixed flour blend of passion fruit peel and albedo with rice was obtained by thermoplastic extrusion. Extrusion was carried out in a single-screw Brabender extruder, model DSE 20 DN, Duisburg, Germany, with mechanical friction extrusion system, screw compression ratio of 3:1, feed rate of 2.5 kg/h, pneumatic cooling system for temperature control in the extrusion barrel, screw speed of 140 rpm, approximate production capacity of 16 kg h^{-1}, and a circular die with a 3 mm outlet thickness. The equipment was fitted with compressed-air cooling for three-barrel sections. The temperature profile of the first zone (60°C) and the second zone (100°C) was fixed, whereas the third zone varied according to the experimental design. The expanded extrudates were dried in a forced-air dryer at 70°C for 4 h. After drying, they were milled in a disc mill with a 1 mm sieve and subsequently in a Perten mill with a 0.8 mm sieve to obtain the mixed extrudate flour used for porridge preparation. The flour mixture was formulated using extrudate flour from passion fruit peel and albedo, skimmed milk powder, flavouring, and sugar in proportions of 50, 20, 10, and 20 g, respectively. Water (300 mL) was heated to 80°C and then added to the mixture, which was homogenized for 1 min to form a paste. Statistical analysis was performed, and differences among means ($p \leq 0.05$) were determined by analysis of variance (ANOVA) followed by Tukey's test. Sensory testing of the porridge formulations with and without flavouring was conducted using the three best formulations (Fm8, Fm12, and Fm13) with 100 panellists, applying a 9-point verbal structured hedonic scale. The data were analysed by ANOVA and Tukey's test ($p \leq 0.05$). Regarding the sensory analysis of the porridge without flavouring **(Figure 1-Test A)**, formulations Fm8 (5.16), Fm12 (4.54), and Fm13 (4.04) showed the lowest acceptability indices. Fm8 was classified as "neither liked nor disliked," whereas Fm12 and Fm13 were classified as "disliked slightly." In the sensory analysis of the porridge with passion fruit flavouring **(Figure 1-Test B)**, formulation Fm13 showed the highest acceptability index (6.08), corresponding to "liked slightly," compared with Fm8 (5.67) and Fm12 (5.76).

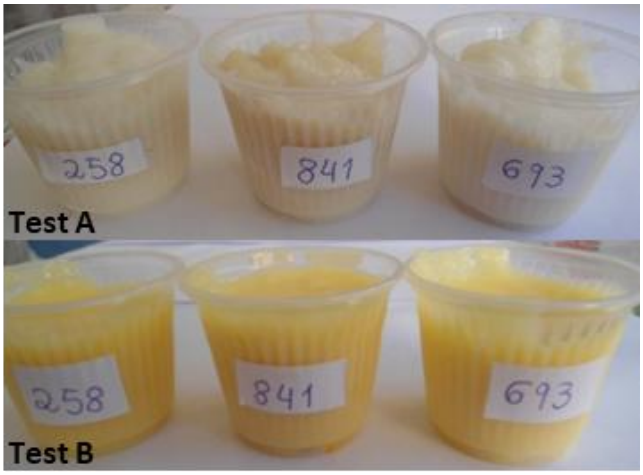


Figure 1 – Test A Coded sample of unflavoured porridge, Test B Coded samples of flavoured porridge

The gluten-free flour blend used in the porridge formulation was obtained by extrusion technology rather than by simple milling and oven drying. Under the conditions studied, extrusion allowed control of raw material moisture content and ensured better texture and dissolution in water or milk for human consumption. The porridge with passion fruit flavouring, particularly formulation Fm13, showed the highest acceptability index.

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Olive pomace valorization through the recovery of bioactive compounds for edible oil fortification

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The olive tree is a crop of great importance, primarily due to the production of olive oil, a product highly valued for its benefits to human health, particularly for its antioxidant properties[1]. The food industry has shown growing interest in byproducts, and the olive oil industry is no exception, with olive pomace being a notable example. After the olive oil extraction process, olive pomace still contains high levels of bioactive compounds, namely phenolic compounds, pigments, and other natural antioxidants, which remain in the byproducts of olive oil production[2]. The valorization of this byproduct represents an opportunity to develop innovative and sustainable solutions, in line with the principles of the circular economy and the reduction of agro-industrial waste.

The efficient recovery of these compounds has been studied to minimize the environmental impact of the olive oil industry and contribute to greater valorization of the sector. In this study, olive oil (cv. Cobrançosa) was first extracted using an Abencor pilot system. The resulting olive pomace was then reprocessed by incorporating different oil formulations (100% olive oil, blends containing 5%, 10%, 20%, and 40% sunflower oil in olive oil, and 100% sunflower oil), to promote the transfer of bioactive compounds retained in the pomace to sunflower oil. Each treatment was performed in quadruplicate, with the original olive oil and sunflower oil used as controls. The oil additions were carried out during the malaxation step, and the mixtures were malaxed for 30 minutes at 25 °C, followed by centrifugation at 3500 rpm in the Abencor pilot system. The recovered oils were subsequently analyzed for quality parameters (free acidity, peroxide value, and specific ultraviolet extinction coefficients), total phenolic compound content, oxidative stability, colorimetric parameters, and descriptive sensory analysis. The results showed that free acidity remained low in all formulations, ranging from 0.17 to 0.34%, indicating that the enrichment process did not substantially affect this quality parameter, although significant differences were observed among formulations ($p < 0.05$). Peroxide value increased from 8.2 meq O_2 kg^{-1} in olive oil to 13.3-14.2 meq O_2 kg^{-1} in the enriched formulations, while K232 and K268 increased from 1.73 and 0.16 to 3.65-3.89 and 0.81-1.02, respectively, indicating higher levels of primary and secondary oxidation products. Total phenolic content was inversely related to the proportion of sunflower oil incorporated, decreasing from 663.3 mg gallic acid kg^{-1} oil in the 5% sunflower oil formulation to 398.4 mg gallic acid kg^{-1} oil in the 40% sunflower oil formulation, while remaining significantly higher than the sunflower oil control (0.0 mg gallic acid kg^{-1}). Similarly, oxidative stability decreased progressively with increasing sunflower oil incorporation, from 5.36 h in the 5% sunflower oil formulation to 3.3 h in the 40% sunflower oil formulation, compared with 14.7 h for olive oil and 2.2 h for sunflower oil. Descriptive sensory analysis showed that olive oil exhibited the highest intensities of green fruitiness (4.5), apple (3.0), tomato (3.6), cabbage (4.6), tomato leaves (4.0), bitterness (3.5), pungency (4.2), complexity (8.6), and persistence (8.6). These attributes progressively decreased with increasing sunflower oil incorporation, reaching 0.0 for most positive sensory attributes and 0.0 for bitterness and pungency in the 40% sunflower oil formulation, whereas sweetness increased from 1.0 in olive oil to 3.6 and 3.7 in the 5% and 10% sunflower oil formulations, respectively. Principal component analysis (Figure 1) explained 85.2% of the total variance (PC1 = 65.0%; PC2 = 20.2%) and clearly discriminated the oil formulations according to their physicochemical and sensory characteristics. Olive oil was associated with higher total phenolic content, oxidative stability, bitterness, pungency, persistence, complexity, and green sensory attributes, whereas sunflower oil was associated with higher K232 and K268 values. The enriched formulations showed a progressive shift from the olive oil to the sunflower oil profile as the proportion of sunflower oil increased, with the 5% formulation remaining the closest to olive oil. Overall, olive pomace proved to be an effective source of bioactive compounds for edible oil fortification. Lower sunflower oil incorporation levels (5-10%) promoted greater phenolic recovery while preserving higher oxidative stability and sensory quality, representing a sustainable strategy for olive pomace valorization and the development of value-added functional oils.

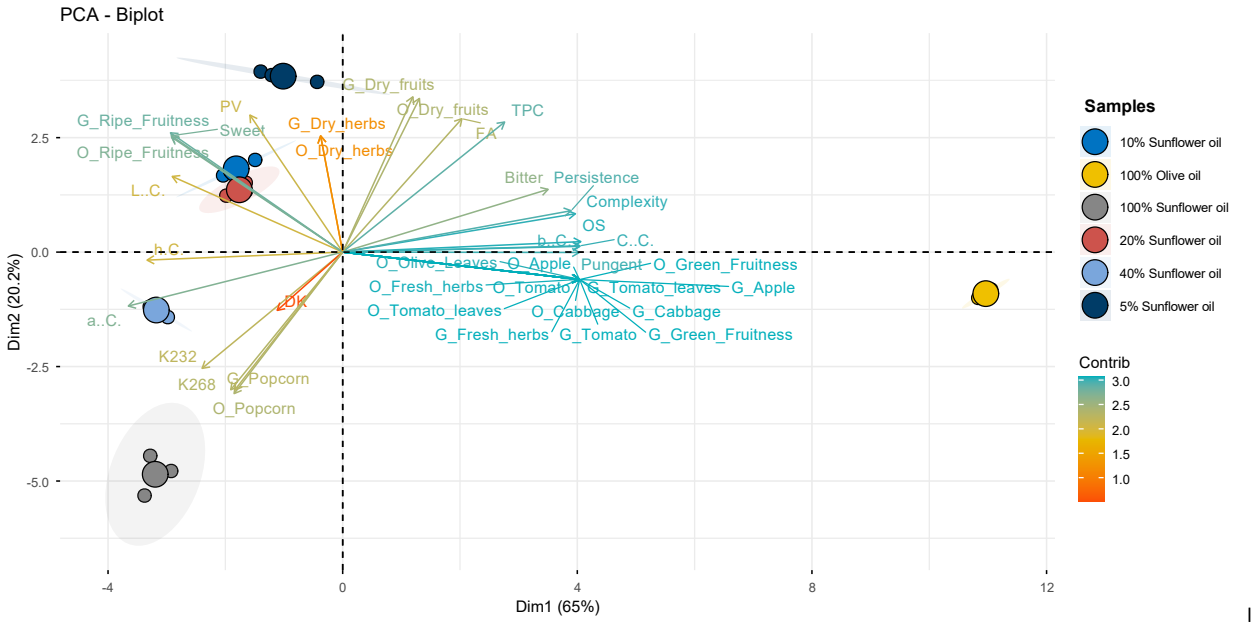


Figure 1 – Principal component analysis (PCA) score plot showing the distribution of the different oil formulations according to their physicochemical and sensory characteristics. PC1 and PC2 explained 65.0% and 20.2% of the total variance, respectively, accounting for 85.2% of the total variance.

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Valorization of Brewers' Spent Grain Using Mechanical Pre-treatments to Extract Soluble Arabinoxylans with Prebiotic Potential

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Brewer's spent grain (BSG), the major by-product of the brewing industry, represents an abundant and underexploited source of arabinoxylans (AX), offering significant opportunities for circular bioeconomy approaches. Although BSG is mainly used as animal feed or disposed of as waste, its valorization into high-value functional ingredients could enhance the sustainability and economic competitiveness of the brewing sector. Arabinoxylan-derived oligosaccharides, namely arabinoxylan-oligosaccharides (AXOS) and xylo-oligosaccharides (XOS), have attracted increasing interest due to their recognized prebiotic properties, promoting gut microbiota modulation and contributing to improved gastrointestinal health, and potential application as functional food ingredients 1. However, the recovery of these biomolecules from BSG remains challenging, as most soluble AX are released into the wort during the brewing process, leaving the majority of insoluble AX within the outer layers of the grain matrix 2. To address these limitations, this work explores a novel pilot-scale solid-liquid extraction technology, Turbex, based on rotor-stator induced turbulence, high-shear forces and cavitation, to enhance extraction efficiency at lower temperatures. To reproduce these mechanical effects at laboratory scale, Ultra-Turrax (UT) and ultrasound-assisted (US) treatments were applied individually and sequentially as pre-treatments prior to enzymatic hydrolysis with endo-xylanase. Initially, BSG was subjected to UT and US to facilitate the removal of glucose from residual starch while promoting disruption of the lignocellulosic matrix. The insoluble fraction was enzymatically hydrolyzed using endo-xylanase to produce arabinoxylan-oligosaccharides and xylo-oligosaccharides, compounds recognized for their prebiotic potential. Process conditions were optimized to maximize extraction yield while increasing the purity of arabinose- and xylose-rich fractions. The sequential pre-treatment combining Ultra-Turrax (1 min, 6500 rpm) and ultrasound (3 min, 40% amplitude) in the presence of 1% (v/v) hydrogen peroxide provided the highest extraction yield (7.6%), recovering 2.3% arabinoxylans with a purity of 29.6%.

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Funding: This work was financially supported by by the Portuguese Science Foundation (FCT).

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Exploring the valorization of agar extraction byproducts through carbohydrate characterization

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Introduction: Agar is a commercially important hydrocolloid obtained from red seaweed. Its extraction process generates large volumes of byproducts that are commonly discarded, representing a significant loss of potentially valuable compounds and an unaddressed challenge for a more circular and economically efficient production chain.

Objective: This study aimed to assess the valorization potential of these byproducts through the characterization of their carbohydrate fraction, providing insights into possible value-added applications.

Methods: Fresh *Gelidium corneum* was pretreated with alkali solution (NaOH 5%, 2 h, 85 °C) and then washed until neutral pH. The alkaline wastewater was dialyzed to remove salts. Further, agar extraction was carried out in the solid residue with water (90 °C) for 2 h. The residue was separated by cloth filtration, and the obtained agar was frozen overnight. After thawing at room temperature, the remaining wastewater was collected, and agar gel was freeze-dried (**Figure 1**). Neutral sugars were analyzed as alditol acetates by gas chromatography and sulphate esters content was determined by turbidimetry [1].

Results: Alkaline wastewater contains around 29% (w/w) of carbohydrates, mainly galactose (Gal) and 3,6-anhydrogalactose (AnGal), 49 and 27 mol%, respectively. Part of the Gal content is likely associated with (iso)floridoside and its oligosaccharide derivatives, compounds synthesized by red seaweed for osmotic regulation. These molecules have attracted increasing interest due to their promising bioactive properties, including prebiotic activity mediated by gut microbiota modulation and promotion of beneficial bacterial growth [2]. The insoluble residue, separated after the agar extraction, corresponded to 27% (w/w) of the initial biomass with a carbohydrate content of 43% (w/w). It is mainly consisted of Gal (45 mol%), AnGal (29 mol%) and sulphates (7 mol%), associated with the presence of agar, and glucose (Glc, 15 mol%) from floridean starch and cellulose. The predominance of agar in this fraction suggests its potential as a source of agarooligosaccharides, which can be obtained by hydrolysis of agar and have been reported to exhibit antioxidant and prebiotic properties [3]. The freeze-thaw wastewater, remaining after thawing and separation of agar gel, has a carbohydrate content of 24% (w/w), similar to the alkaline wastewater, but is composed mainly of Gal and Glc (47 and 39 mol%, respectively). The obtained agar has a purity of 77% (w/w) and low sulphates content (2 mol%).

Conclusion: Overall, these results highlight the valorization potential of agar extraction byproducts, indicating that valuable carbohydrate fractions can be recovered and further explored for food applications, including as functional ingredients with rheological or bioactive properties.

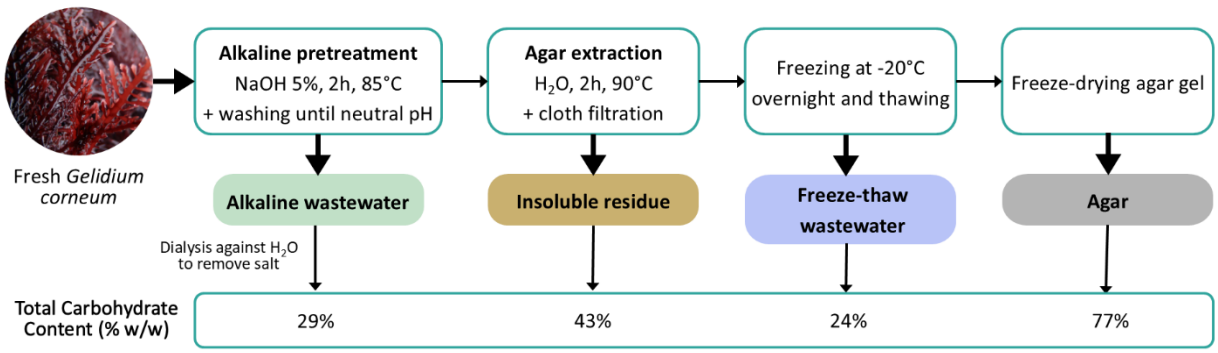


Figure 1 - Agar extraction process flow from Gelidium corneum seaweed, obtention and analysis of byproducts.

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Sustainable extraction techniques for bioactive antioxidants from Ulva *rigida* and *Sargassum*: comparison of seaweeds and assessment of the optimal approach

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This study aimed to compare a brown (Sragassum) and a green (Ulva *rigida*) seaweed in its antioxidant profile, as well as comparing diferent extraction methods using water as the extracting solvent. The two seaweeds were subjected to solid–liquid extraction (SLE), where the extraction conditions biomass (g):solvent (mL) ratio and time remained constant. The dry (45°C) seaweed was ground to 0.180-0.315 mm mesh and rehydrated in a 1:20 ratio seaweed:water in all experiments. The mixtures were subjected to orbital water extraction (OWE), at 125 rpm and 45°C (OWE45); water extraction with stirring speed of 2000 rpm at 20°C (WE20); water extraction with glass bead beating size[1], stirring speed of 2000 rpm at 20°C (GBB1-20); water extraction with glass bead beating size[2], stirring speed of 2000 rpm at 20°C (GBB2-20); and ultrasound-assisted extraction (UAE45) at 45°C. **Figure 1** shows the TPC, DPPH, ABTS and FRAP values obtained from the extracts of Ulva and Sargassum expressed in µg of GAE/g d.w., µg Trolox/g d.w., respectively, using the five different extraction methods. For the green seaweed there are significant differences (p = 0,007) between orbital water extraction (125 rpm, OWE45) and the water extraction with stirring speed of 2000 rpm at 20°C (WE20), with the highest TPC value of 1320 µg GAE/g d.w. seaweed for the later, with lower values for all the other extraction methods. The extracts obtained from the brown algae, *Sargassum*, present the highest TPC value at 1715 µg GAE/g d.w. seaweed, with significant differences to the other methods (p = 0,003), when using ultrasound assisted extraction (UAE45) at 45°C. Relative to the antioxidant concentration measured by the FRAP test, there are significant differences between the two bead beating methods. The method using smaller glass beeds (GBB1-20) produces Sarg assume extracts with higher FRAP value, 1460 µg TE/g d.w. compared to 1090 µg TE/g d.w seaweed for the bigger glass beeds (GBB2-20). The glass beads have a strong effect in cell disruption allowing the compounds to leak to the water solution. The size of these beeds should be adapted to the size of the particles that are being disrupted[2].

Comparing the two seaweeds, in all extraction methods, there are significant differences in the antioxidant content measured by the FRAP test, and the Sargassum has higher values than Ulva *rigida* (**Figura 1**).

Depending on the extration method, it is possible to maximize the bioactive antioxidants in the extracts, and in general, the Sargassum seaweed has higer potential than Ulva *rigida* to produce extracts more concentrated with antioxidants. Water is a good extracting solvent for bioactive antioxidants from these algae.

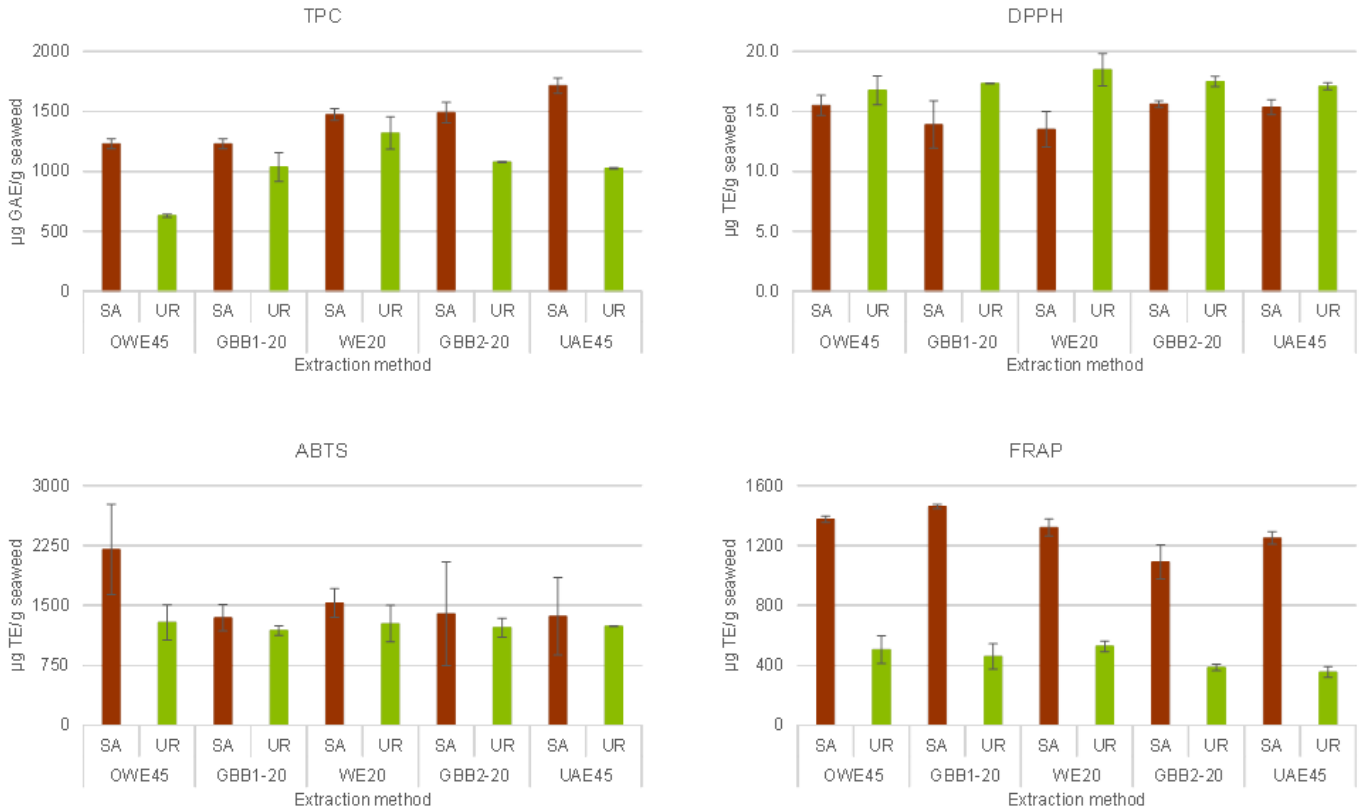


Figure 1 - Shows the TPC, DPPH, ABTS and FRAP values of the Ulva and Sargassum expressed in µg of GAE/g d.w., µg Trolox/g d.w., respectively, for the extracts obtained from the five different extracting methods.

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Sequential extraction strategies to recover organic acids and phenolic compounds from garlic by-products with food preservative potential

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The valorization of food-industry by-products is a promising strategy envisaging the obtention of natural ingredients for sustainable food preservation systems, including food coatings. Garlic (Allium sativum L.) production and processing generate non-edible by-products that are often discarded despite containing biologically active compounds, particularly organic acids and phenolic compounds.1 Organic acids may contribute to food microbiological stability by acidifying the medium and interfering with microbial metabolism,2 while phenolic compounds are primarily associated with antioxidant activity, free radical scavenging, lipid oxidation hindering and quality deterioration.3 The objective of this study was to evaluate the potential of garlic by-products as a source of natural compounds for food preservation by comparing three extraction techniques for sequentially recovering organic acids and phenolic compounds from the same biomass.

In the first stage, organic acids were extracted using an aqueous solvent. The resulting solid residue was redried in an oven at 40 °C for 12 hours to standardize the matrix, prior to phenolic extraction with a 25% hydroethanolic solution. Three extraction techniques were evaluated: maceration at 25 °C, ultrasound-assisted extraction (275 W for organic acids and 130 W for phenolic compounds), and microwave-assisted extraction (1000 W for organic acids and 665 W for phenolic compounds). All techniques were conducted in duplicate for 23.5 minutes with a solid/liquid ratio of 1 g/30 mL. The fractions were analyzed by HPLC-DAD for organic acid quantification and chromatographic analysis of phenolic compounds. Additionally, the hydroethanolic fraction was characterized using the Folin-Ciocalteu assay, with the results being expressed as gallic acid equivalents (GAE), and antioxidant activity was determined using the DPPH method. The yield of both fractions was also quantified.

For the aqueous fraction, maceration resulted in the highest extraction yield, being also the most efficient technique for the recovery of organic acids, reaching 36 ± 9 g/100 g dry weight (dw). This fraction contained oxalic, malic, and citric acids, suggesting that the higher yield obtained by maceration was associated with the greater organic acid recovery. In the hydroethanolic fraction, ultrasound-assisted extraction produced the highest yield, while microwave-assisted extraction produced the highest response in the Folin-Ciocalteu assay (16.03 ± 0.01 mg GAE/g extract). However, chromatographic analysis revealed larger peak areas for phenolic compounds in the macerated extract, indicating a higher chromatographic response for these compounds. This difference may be partly explained by the low specificity of the Folin-Ciocalteu assay, which can also respond to non-phenolic reducing compounds. Yet, a higher response in this assay cannot be directly attributed to a greater content of phenolic compounds. Regarding the antioxidant activity, the macerated extract presented the lowest EC_{50} value, 1.22 ± 0.01 mg/mL, among the evaluated extraction techniques, holding the highest antioxidant efficiency. This antioxidant performance supports further evaluation of the extract for use in active food coatings, where it will hinder the oxidative reactions associated with food deterioration. Sequential extraction enabled the recovery of different chemical compound classes from the same garlic by-product biomass, supporting a more complete and sustainable use of this residue. Although the most suitable extraction technique varied depending on the target compound class, maceration showed the most consistent overall performance, with the highest organic acid recovery, a larger chromatographic response for phenolic compounds, and the lowest EC_{50} value in the DPPH assay. These results support further evaluation of garlic-derived fractions as functional agent for active food coatings and preservation systems.

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Bioactive Profiling of Parsley and Coriander Anatomical Fractions for Sustainable Functional Food Applications

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Aromatic herbs such as parsley (Petroselinum crispum L.) and coriander (Coriandrum sativum L.), belonging to the Apiaceae family, are recognised as important sources of bioactive compounds with nutritional and functional relevance1. Rich in phenolic compounds, flavonoids, chlorophylls, and carotenoids, these herbs have attracted growing interest for their potential application in functional foods and sustainable food systems2. Some studies focus specifically on the polyphenol content of coriander extracts and on the comparative antioxidant properties of parsley and coriander, highlighting their importance as protective plant-based matrices3. However, despite their widespread culinary use, only the leaves are commonly valorised, while stems are frequently discarded as food waste, contributing to the underutilisation of nutritionally valuable plant biomass. In the context of sustainable food production and circular bioeconomy strategies, the comprehensive exploitation of edible plant materials has become increasingly important. Therefore, understanding the distribution of bioactive compounds between different anatomical fractions is essential to support whole-plant utilisation approaches capable of reducing waste and maximising nutritional value.



Figure 1 – Coriander (Coriandrum sativum L.) and parsley (Petroselinum crispum L.) used for bioactive profiling.

This study aimed to characterise and compare the biochemical composition of fresh leaves and stems of parsley and coriander by quantifying photosynthetic pigments (chlorophylls and carotenoids), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC). The objective was to evaluate the bioactive potential of each anatomical fraction and assess their contribution to sustainable whole-plant valorisation.

The results revealed a significant biochemical differentiation between leaves and stems in both species. Regarding pigmentation, coriander leaves exhibited the highest pigmentation, recording peak values for chlorophyll a (20.94 µg/mL), carotenoids (4.85 µg/mL), and an exceptional TFC of 2.72±0.23 mg CE/g. In contrast, parsley leaves showed a more balanced phenolic profile with a TPC of 2.01±0.02 mg GAE/g. Notably, stems, commonly regarded as culinary food by-products, presented considerable functional potential. Coriander stems proved to be surprisingly rich in phenolics and flavonoids, with a TPC of 2.07±0.06 mg GAE/g and a TFC of 2.10±0.03 mg CE/g, significantly outperforming parsley stems in these categories.

These findings demonstrate that although leaves constitute the primary reservoir of photosynthetic pigments, stems, particularly those of coriander, represent an important source of phenolic and flavonoid compounds with potential antioxidant activity. The integral utilisation of these herbs may therefore contribute to maximising the intake of bioactive compounds while simultaneously reducing food waste and promoting more sustainable food practices. This study highlights the relevance of whole-plant approaches in the valorisation of aromatic herbs and supports their application as functional ingredients in food and nutrition systems.

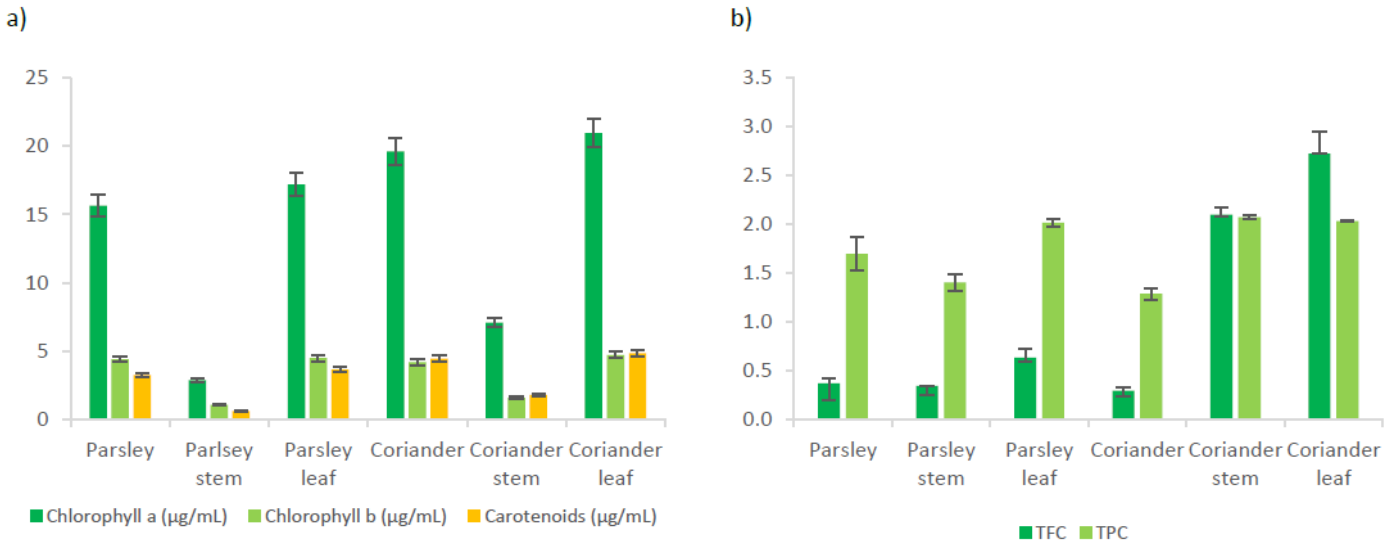


Figure 2 – Comparative bioactive composition of parsley and coriander fractions: (a) chlorophyll a, b and carotenoid contents; (b) total phenolic content (TPC) and total flavonoid content (TFC).

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Tryptophan: the importance of food chemistry focusing the delicate balance between the cell requirements and metabolic impairments

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Tryptophan (Trp) is widely considered as an essential amino aromatic acid to humans, since is not produced by human cell metabolism.

It is widely found in meat, eggs, dairy products, beans, soybeans, grains, eggs, tuna, salmon, tofu, chocolate, banana or nuts. Furthermore, several gut bacteria can produce Trp. Hence, Trp is abundant in omnivorous or vegetarian (and vegan) diets.

Nowadays, it is well documented in literature that the impairment in (at least one of) the four metabolic pathways of Trp are very common and often a cause for several chronical diseases, as tryptophan metabolism is crucial for physiological homeostasis. Trp is the serotonin (and melatonin) precursor, and so, rich food in Trp is recommended in long depression status patients. Also, its metabolites influence immune regulation, cellular energy production, and closely interact with the gut microbiota, promoting several physiological amendments.

Dysregulation in tryptophan's metabolic pathways has been linked to a variety of diseases, including metabolic disorders such as obesity and diabetes, neuropsychiatric conditions like depression, schizophrenia or bipolar disorder or neurogenerative diseases, and autoimmune diseases such as multiple sclerosis and inflammatory bowel disease (IBD).

For most of the Trp-linked metabolic disorders, the regulation of the Trp content in ingested food is crucial, and low Trp diets are recommended. Moreover, the uptake of Trp is dependent of the presence of other amino acids, namely lysine (Lys). Nevertheless, nowadays, in food industry Trp rich diets, present everywhere, mainly in pastries, restaurants, street food places and supermarkets, over difficulties the health management in patients with these disorders linked to Trp.

Hence, with the recent advances in the knowledge of the physiological and mental effects of Trp, it is mandatory that food industry, along with food chemistry, change their way of thinking and introduce new and appellative food products to supply healthy and interesting food to a large amount of people that present impairments in the Trp metabolic pathways, in good agreement with recent FoodOmics concept.

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Assessment of Pulsed Electric Field Processing for the Microbial Inactivation of Escherichia coli in Raw Bovine Milk

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Pulsed electric field (PEF) technology has emerged as a promising non-thermal preservation approach for raw bovine milk, offering enhanced microbial safety while preserving its nutritional and physicochemical quality compared with conventional thermal processing. The application of short, high-voltage electric pulses enables the inactivation of pathogenic and spoilage microorganisms with minimal thermal damage; however, its efficiency is dependent on microbial specific resistance and strongly influenced by processing parameters, including electric field strength, pulse characteristics and treatment time. Consequently, process optimization is essential to maximize microbial inactivation and support industrial implementation in the dairy sector, in particular when low-intensity PEF treatments are to be used along with lower pasteurization temperatures to achieve adequate log reductions of pathogenic bacteria.

This study evaluated the microbial inactivation efficiency of PEF against *Escherichia coli* inoculated in raw bovine milk.

Experiments were performed using a laboratory-scale EPULSUS®-LPM1A-10 pulsed electric field (PEF) system operating at 10 kV. Two treatment conditions were tested: PEF1 (10 µs pulse width, 27 Hz) and PEF2 (10 µs pulse width, 39 Hz). The survival of *Escherichia coli* was evaluated before and after treatment by enumeration on Tryptone Bile X-Glucuronide (TBX) agar. Sublethal injury was assessed by comparing counts on Tryptic Soy Agar (TSA) and TSA supplemented with 5% NaCl.

Both PEF treatments resulted in microbial reductions, although the level of inactivation was strongly influenced by the enumeration medium used. The highest reduction was achieved with PEF1 when cells were enumerated on TSA, reaching 1.33 log CFU mL^{-1}. However, significantly lower reductions were observed on the selective medium TBX, with values of 0.21 and 0.16 log CFU mL^{-1} for PEF1 and PEF2, respectively. Likewise, counts obtained on TSA supplemented with 5% NaCl showed only limited reductions (0.18–0.21 log CFU mL^{-1}). The differences among media may suggest the presence of sublethally injured cells capable of recovering under non-selective conditions but exhibiting impaired growth under selective or stress-inducing environments.

However, TSA supplemented with 5% NaCl did not show the increased sensitivity typically expected for the detection of sublethally injured cells. The relatively small differences between TSA and TSA + 5% NaCl indicate that surviving cells may retain a substantial capacity to tolerate osmotic stress, or alternatively, that the extent of PEF-induced membrane damage was insufficient to significantly impair recovery.

These results highlight the complexity of microbial stress responses to PEF processing and underscore the need for complementary methodological approaches for accurate assessment of sublethal injury. Furthermore, they suggest the possibility that exposure to sublethal PEF conditions may trigger cellular protective mechanisms, such as the upregulation of chaperones/HSPs, which could not only mitigate PEF-induced damage but also contribute to cross-protection against osmotic stress imposed by the presence of 5% NaCl.

Overall, PEF processing at 10,000 V resulted in measurable reductions of *Escherichia coli* in raw bovine milk, with the highest reduction observed under the 10 µs/27 Hz treatment condition. Although the inactivation levels were lower than those reported for higher field strengths, the results are consistent with previous studies indicating that Gram-negative bacteria such as *E. coli* display moderate sensitivity to low-intensity PEF treatments. Further research incorporating higher energy inputs, as well as complementary physiological and structural analyses, are required to optimize process conditions, improve microbial inactivation, and better understanding of the mechanisms underlying sublethal injury in dairy matrices.

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Investigating the use of FT-RAMAN to differentiate commercial vinegars.

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Vibrational spectroscopy is a rapid, non-destructive analytical technique widely used to characterise and differentiate complex food matrices. In the context of food authentication, it offers a useful approach for distinguishing commercial vinegars based on their molecular composition and functional group signatures [1,2].

This work aimed to explore the ability of FT-Raman to differentiate commercial vinegars, supported by conventional physicochemical measurements, to assess their chemical variability and classification potential.

The major components of vinegars are acetic acid and water, but its composition, rich in other compounds, is greatly influenced by the raw materials used and by the production process, such as submerged fermentation in reactors or traditional ageing in wooden barrels. In this work, commercial vinegars produced from different raw materials, including wine, apple, malt, fruit, honey, cider, cereals and balsamic substrates, were evaluated. A total of 34 commercial vinegar samples were analysed, with measurements taken for standard physicochemical parameters such as pH, total acidity, °Brix, colour, electrical conductivity and total phenolic content.

The spectra of vinegar samples were obtained by FT-RAMAN with a spectrometer (Bruker, MultiRAM, Germany), equipped with a Ge Diode detector, an integrated 1064 nm and Nd:YAG laser with a maximum output power of 500 mW. The spectra were collected with 1000 scans per spectrum at a spectral resolution of 4 cm^{-1} in the wavenumber range from 3500 to 70 cm^{-1}.

Distinct spectral patterns were observed across vinegar types. The IR profiles were characterised by absorption bands corresponding to O–H, C=O and C–O functional groups [3]. Fruit-derived vinegars generally exhibited more complex spectral features, consistent with the presence of sugars and phenolic compounds, whereas distilled or synthetic samples showed simpler profiles.

The physicochemical data showed clear variability, with pH values ranging from 2.1 (cereal vinegar) to 3.4 (fruit vinegar) and significant differences in °Brix and acidity, indicating the samples variability. Total phenolic content ranged from 41.8 mg EAG/ L of vinegar (cereal vinegar) to 3289.4 mg EAG/ L of vinegar (Fruit vinegar), reflecting variations in bioactive compounds. Moreover, colour measurements demonstrated consistent differences in luminosity and chromatic properties.

Principal Component Analysis (PCA) of the FT-Raman data enabled effective separation of samples by their compositional characteristics and also by the sugar content (related to °Brix) to supporting classification by origin and production type. An independent PCA performed only with fruit vinegars show that the FT-Raman is also able to distinguish some raw materials.

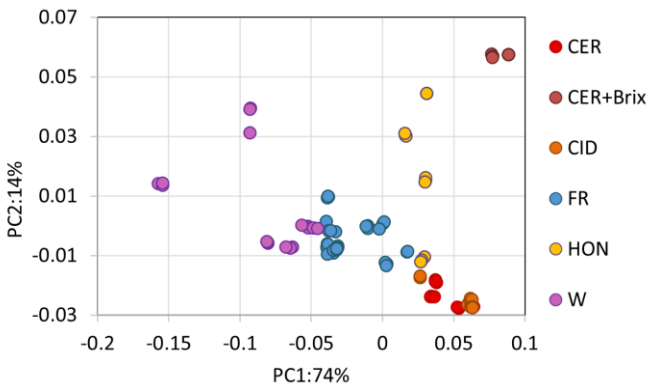


Figure 1 - Principal Component Analysis (PCA) performed on pre-processed Raman spectra, using PC1 and PC2 to assess clustering and discrimination between samples. CER – Cereal vinegar; CER+Brix – Sweetened Cereal vinegar; CID – Cider vinegar; FR – Fruit vinegar; HON – Honey vinegar; W – Wine vinegar.

The results of this study revel a higher differentiation between the characteristics of the different commercial vinegar. Combination of FT-Raman spectroscopy and physicochemical analysis proved to be a suitable strategy for differentiating vinegar, with clear potential for use in authenticity assessment and quality control.

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Macroalgae-Enriched Chickpea and Almond Spreads as Sustainable Functional Foods: Nutritional Potential and Ingredient Selection

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The increasing demand for plant-based foods has stimulated the search for novel ingredients capable of simultaneously improving nutritional quality, functionality and sustainability[1]. Among the most promising resources, macroalgae have attracted growing attention due to their richness in proteins, dietary fibre, minerals and bioactive compounds, as well as their low environmental footprint[2]. The incorporation of macroalgae into food products may contribute to the development of innovative functional foods while supporting the transition towards more sustainable dietary patterns. In this context, vegan spreads represent an attractive food matrix due to their versatility, consumer acceptance and potential to serve as carriers of bioactive ingredients.

This work evaluated the suitability of three edible macroalgae species from the Portuguese coast, *Fucus vesiculosus*, *Ulva rigida* and *Gracilaria gracilis*, for application in chickpea- and almond-based vegan spreads. The selected species were characterised regarding their nutritional composition and extraction potential, aiming to identify their contribution to the development of nutritionally enhanced and functionally differentiated products.

Significant differences were observed among the species studied. *G. gracilis* exhibited the highest protein content (28.5%), highlighting its potential as a protein-enriching ingredient. In contrast, *U. rigida* showed the highest dietary fibre content (6.2%), suggesting potential benefits for digestive health and texture enhancement. *F. vesiculosus* presented a distinctive nutritional profile, characterised by a considerable mineral content. Extraction studies revealed substantially higher yields for aqueous extraction (32.5–48.5%) compared with ethanol (5.0–7.5%) and acetone (2.0–4.0%), indicating the predominance of water-soluble compounds and supporting the use of environmentally friendly extraction approaches.

The combination of chickpea, almond and macroalgae offers complementary nutritional advantages. Chickpea provides plant protein, complex carbohydrates and structure, while almonds contribute healthy lipids, flavour and consumer familiarity. The incorporation of macroalgae further enhances the nutritional profile by supplying additional proteins, minerals, dietary fibre and bioactive compounds. The incorporation of macroalgae significantly influenced the spreadability properties of chickpea-based spreads ($p < 0.05$). Macroalgae incorporation significantly influenced the spreadability of both chickpea- and almond-based spreads. In chickpea formulations, spreadability work increased from 18.5 N·s in the control to 21.0, 22.3 and 24.5 N·s following the incorporation of *G. gracilis, U. rigida* and *F. vesiculosus*, respectively. A similar trend was observed for almond-based spreads, with values increasing from 25.0 N·s in the control to 28.4, 29.7 and 31.6 N·s, respectively. The higher spreadability work observed in almond-based formulations suggests the formation of a denser and more cohesive matrix, likely resulting from interactions between almond lipids, proteins and macroalgal polysaccharides. Among the tested species, *F. vesiculosus* produced the strongest structuring effect, probably due to its high content of alginates and fucoidans, whereas *G. gracilis* exerted a more moderate influence. These results demonstrate that Portuguese macroalgae can act not only as nutritional enhancers but also as multifunctional ingredients capable of improving the technological performance and stability of plant-based spreads. Furthermore, the valorisation of marine biomass contributes to circular bioeconomy strategies and promotes the diversification of plant-based foods available to consumers. These findings provide a scientific basis for the development of innovative spread formulations combining legumes, nuts and macroalgae as sustainable ingredients for future food systems.

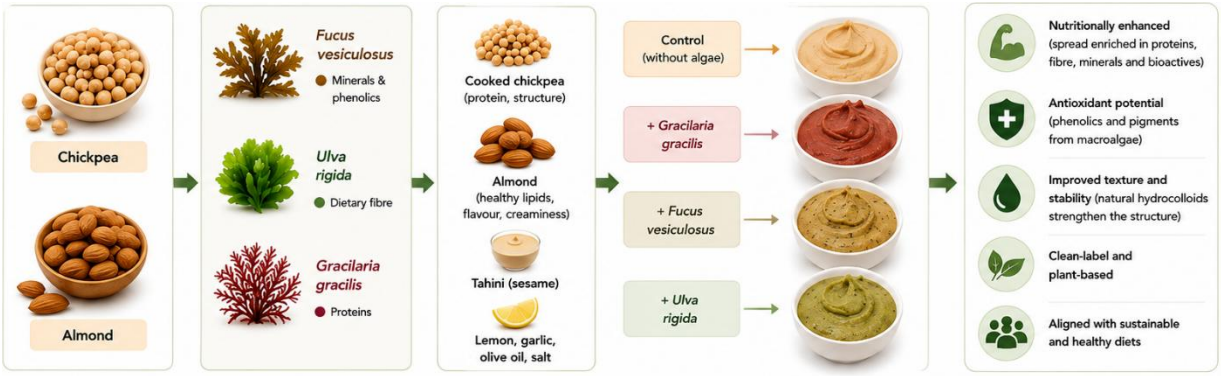


Figure 1 - Overview of the development of chickpea–almond vegan spreads enriched with Portuguese macroalgae. The scheme illustrates the selected macroalgae species, formulation strategy, nutritional and functional contributions, and the expected sustainability benefits of the resulting products.

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Gluten-free, egg-free pancakes enriched with sweet potato flour: from technology to bioactive bioaccessibility

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The growing demand for gluten-free products with improved nutritional and functional profiles has driven interest in non-conventional plant-based flours. Sweet potato (*Ipomoea batatas* L.) stands out for its agronomic resilience and varietal diversity; orange-fleshed (*Bellevue*) and purple-fleshed (*NP1648*) cultivars are rich in carotenoids and anthocyanins, respectively, making them promising functional food ingredients[1]. However, the technological behaviour of sweet potato flour in gluten-free matrices and the post-digestion retention of its bioactive compounds following processing remain underexplored[2]. To address this knowledge gap, two gluten-free, egg-free pancake powder formulations incorporating orange- and purple-fleshed sweet potato flours, rice flour, certified gluten-free oat flour, and flaxseed as an egg substitute were developed. Samples were characterised at three processing stages (flour, powder mix, and pancake) for nutritional composition, bioactive compounds (carotenoids and anthocyanins, dry-weight basis), antioxidant activity (DPPH and FRAP, dry-weight basis), techno-functional properties (water/oil absorption capacities), and texture (TPA). Consumer acceptance was assessed by hedonic sensory evaluation (n = 61) against a homemade control (wheat flour + egg) and a commercial reference. Post-digestion retention of carotenoids and anthocyanins was assessed using the INFOGEST 2.0 static *in vitro* digestion protocol in raw flours and cooked pancakes reconstituted with whole cow's milk (3.5% fat) and an oat-based plant drink. Orange-fleshed sweet potato flour could be incorporated up to 20% before batter consistency and pancake structure were compromised, whereas the purple-fleshed formulation tolerated incorporation levels up to 60%, yielding a product with higher bioactive content. Carotenoid concentration (75.0 ± 2.6 mg β-carotene/100 g db) declined ($p < 0.05$) following formulation and cooking. Anthocyanin levels (*ca*. 17.6 ± 1.8 mg cy-3-glc/100 g db) were maintained throughout processing. DPPH radical-scavenging activity increased progressively across processing stages for both formulations, most markedly in purple sweet potato pancakes (nearly sixfold relative to raw flour). FRAP values increased similarly for the purple formulation, whereas no significant difference was observed in the orange formulation. Following ingredient blending, water absorption capacity decreased slightly in the orange formulation, and oil absorption capacity increased ($p < 0.05$) in both formulations. Nutritionally, both sweet potato formulations contained less energy and fat than the homemade control (≈226 kcal, 2.8 g fat). The orange formulation provided ≈180 kcal, 12.4 g protein, and 0.3 g fat, while the purple formulation provided ≈169 kcal, 9.5 g protein, and 0.8 g fat (per 100 g, wb). Orange sweet potato pancakes were significantly softer (hardness: 542.8 ± 189.3 g) than purple (1440.2 ± 221.0 g) and control (1227.4 ± 185.8 g) formulations, while cohesiveness and resilience were comparable between the orange and control samples. Hedonic evaluation revealed positive consumer acceptance for both sweet potato formulations, with overall liking and texture scores comparable to those of the commercial reference. Post-digestion carotenoid retention was markedly higher in pancakes reconstituted with oat-based plant drink (78.2%) than in pancakes reconstituted with whole milk (14.9%), reflecting the hydrophobic nature of these pigments and their tight association with the food matrix. Apparent anthocyanin recovery after digestion reached 99.3% in pancakes reconstituted with whole milk and exceeded initial values (139.5%) in pancakes reconstituted with oat-based plant drink, likely reflecting the release of matrix-bound forms. DPPH activity increased substantially after digestion in all pancake samples (146–262%), whereas FRAP increased in purple formulations but decreased in orange ones, indicating cultivar-dependent antioxidant release patterns. These findings demonstrate that gluten-free, egg-free pancakes enriched with sweet potato flour are technologically viable, offer a favourable nutritional profile, and are well accepted by consumers. Gastrointestinal digestion enhanced the antioxidant activity of both formulations; however, the magnitude of bioactive release was strongly dependent on both the chemical nature of the compounds and the reconstitution vehicle, underscoring the pivotal roles of these factors in developing gluten-free foods with enhanced bioactive potential.

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Whey2BioMelanin: Hydrolyzed Whey as an Alternative Substrate for Melanin Production by *Pseudomonas putida* ESACB 191

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Lactic acid bacteria (LAB) are of considerable interest because of their ability to produce a wide range of metabolites with significant technological value, including antimicrobial compounds, functional metabolites, antioxidants, and enzymes. In addition to their well-recognized probiotic potential for the development of functional foods, LAB also represent promising microbial cell factories for the biosynthesis of compounds with technological and industrial relevance.

This study focuses on the characterization of several wild type *Weissella cibaria* strains, aiming to identify metabolites of biotechnological interest, evaluate their probiotic potential, and assess key safety parameters to support their future application in both functional foods and biotechnological processes.

Seven wild-type *W. cibaria* strains from the Applied Microbiology Unit (UMA/UnIPVC) were evaluated for their safety and functional probiotic potential. Phenotypic characterization and culture optimization were performed using classical culture-dependent microbiological methods. Exopolysaccharide (EPS) production was assessed and characterized by TLC, FTIR, and HPLC. Antioxidant activity (ABTS, DPPH, TPC), enzymatic activities (amylase, protease, lipase, urease, lecithinase, decarboxylase, and acid phosphatase), antimicrobial activity (spot-on-lawn assay), cholesterol removal (OPA method), and tolerance to NaCl, acidic conditions, and bile salts were evaluated. Probiotic-related properties, including cell surface hydrophobicity, autoaggregation, and co-aggregation, were also determined. Safety assessment comprised the evaluation of virulence-associated traits, including gelatinase, DNase, hemolytic activity, lipase, and biogenic amine production.

The strains exhibited antimicrobial activity against selected pathogens, as *Listeria monocytogenes*, through a contact-dependent mechanism, with no evidence of bacteriocin production in cell-free culture supernatants. All strains showed high tolerance to bile salts, significant cholesterol-removal capacity, and strong proteolytic activity. Cell adhesion assays demonstrated high co-aggregation ability with Escherichia coli and Salmonella enterica, suggesting a potential role in competitive exclusion. Safety assessment confirmed the absence or only minimal expression of virulence-associated traits, including gelatinase, lipase, DNase, haemolytic activity, and biogenic amine production. Six of the seven strains produced substantial amounts of very high molecular weight exopolysaccharides (EPS), tentatively identified as α-(1→6)-dextran, which also exhibited strong antioxidant activity.

Considering all strains tested, the W. cibaria PFM1679 strain, combining antimicrobial efficacy, adhesion properties, EPS production, and confirmed safety, emerges as the most suitable candidate for probiotic food formulations and further evaluation.

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Safety Assessment and Probiotic Attributes of *Weissella cibaria*: A Multi-Strain Evaluation for Functional Food Applications

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The global shift towards sustainable production models has intensified the search for naturally derived pigments as alternatives to synthetic colorants, particularly for food, cosmetic, and biomedical applications. In parallel, the dairy industry generates substantial volumes of whey — a protein-rich by-product whose accumulation poses both environmental and economic challenges. The valorisation of whey within a circular bioeconomy framework, by directing it towards high-value microbial bioprocesses, represents a compelling strategy that simultaneously addresses waste management and the demand for bio-based compounds [1].

Melanins are dark-coloured biopolymers with exceptional physicochemical stability, broad-spectrum antioxidant activity, and growing relevance across food, cosmetic, and biomedical sectors. Our group previously demonstrated that *Pseudomonas putida* ESACB 191, a strain originally isolated from goat cheese rind, is capable of producing extracellular eumelanin in tyrosine-supplemented media[2]. However, dependence on purified L-tyrosine as a biosynthetic precursor represents a cost barrier to large-scale production. Given that whey proteins are naturally rich in tyrosine residues, targeted pre-treatment strategies to enhance amino acid bioavailability may offer a viable and cost-effective alternative to conventional synthetic media.

This study evaluates the potential of sweet whey as an alternative substrate for melanin bioproduction by P. putida ESACB 191. Two pre-treatment strategies — thermal denaturation and enzymatic hydrolysis — are being compared for their capacity to release tyrosine and other aromatic amino acids from whey proteins, thereby promoting melanin biosynthesis. The performance of the resulting hydrolysate-based media is being benchmarked against conventional tyrosine-supplemented cultures in terms of pigment yield and production efficiency.

Melanin production assays follow previously validated cultivation protocols for P. putida ESACB 191[2]. Pigment is recovered from cell-free supernatants by acid precipitation, followed by purification and lyophilisation. Structural characterisation is carried out by Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR), with spectra compared against previously reported bacterial eumelanin profiles and commercial melanin standards to confirm the identity and purity of the produced pigment.

The Whey2BioMelanin approach proposes a dual-benefit strategy: reducing dependency on costly purified amino acid precursors while simultaneously valorising a dairy industry by-product. Preliminary results will be presented at the conference, providing initial evidence on the capacity of hydrolysed whey to sustain melanin biosynthesis. The findings are expected to advance the understanding of the relationship between whey protein hydrolysis and pigment biosynthesis, contributing to the design of scalable, sustainable bioprocesses for naturally derived colorant production aligned with current circular economy goals[3].

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Closing the Agri-Food Loop: Ultrasound-Assisted Recovery of Bioactive Compounds from Bell Pepper Plant By-Products

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The growing demand for sustainable agricultural practices and the increasing accumulation of crop residues have intensified the search for innovative valorization strategies aligned with circular bioeconomy principles. Agricultural side streams are increasingly recognized as rich sources of bioactive compounds that can be valorized into high-value products. In this context, the implementation of efficient green extraction technologies is essential to maximize the recovery of these compounds while minimizing solvent consumption, processing time, and environmental impact. Conventional extraction techniques, such as maceration, are widely used for this purpose but are often associated with long extraction times, high solvent consumption, and limited extraction efficiencies. In contrast, emerging green extraction technologies have gained increasing attention owing to their ability to enhance mass transfer, accelerate extraction, and improve the recovery of bioactive phytochemicals[1,2]. Among these technologies, ultrasound-assisted extraction (UAE) has emerged as an efficient approach, exploiting acoustic cavitation to disrupt plant cell structures and facilitate the release of intracellular compounds. Bell pepper (*Capsicum annuum L.*) cultivation generates substantial amounts of end-of-cycle plant biomass that remains largely underutilized despite being a rich source of phenolic compounds and other antioxidant phytochemicals[3]. In this context, this study aimed to optimize the UAE process for the recovery of bioactive compounds from end-of-cycle bell pepper plant by-products. To optimize the extraction process, a central composite design coupled with response surface methodology (RSM) was implemented using ultrasound amplitude (20–100%) and ethanol percentage (0–100%, v/v) as independent variables. Extraction yield, total phenolic content, and antioxidant activity (DPPH scavenging activity, ABTS radical cation scavenging activity, and crocin bleaching inhibition capacity) were used as response variables to determine the optimal extraction conditions for maximizing bioactive compound recovery while minimizing solvent consumption and processing time. Although the analyses are still ongoing, the optimized extraction conditions identified by the response surface models will be experimentally validated by comparing predicted and experimental responses. The extract obtained under the optimized conditions will then be characterized to identify and quantify individual phenolic compounds. Overall, this work is expected to establish an optimized green extraction process for obtaining phenolic-rich antioxidant extracts from bell pepper plant by-products, supporting their sustainable valorization for future agri-food applications.

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Waste-to-Value Innovation: Microwave-Assisted Extraction of Bioactive Compounds from End-of-Cycle Eggplant Biomass for Safe and Sustainable Valorization

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The growing demand for sustainable and resource-efficient production systems has intensified the search for innovative approaches to valorize agricultural side streams within the framework of a circular bioeconomy. Plant by-products are increasingly recognized as rich sources of bioactive compounds that can be converted into high-value ingredients for agricultural, pharmaceutical, and cosmetic applications[1]. Eggplant (Solanum melongena L.) cultivation generates substantial amounts of end-of-cycle plant biomass that remains largely underutilized despite being rich in phenolic acids, flavonoids, and other antioxidant phytochemicals[3]. In this context, the implementation of efficient green extraction technologies is essential to maximize the recovery of these compounds while minimizing solvent consumption, processing time, and environmental impact. Among these technologies, microwave-assisted extraction (MAE) has emerged as a sustainable approach that promotes rapid dielectric heating of the solvent–matrix system, enhances cell wall disruption, and facilitates the release of intracellular compounds[2]. MAE improves extraction efficiency while reducing processing time, making it a sustainable alternative for recovering bioactive compounds from plant biomass. Accordingly, this study aimed to optimize MAE conditions to recover bioactive compounds from end-of-cycle eggplant aerial biomass. Before MAE optimization, the raw plant material was screened for pesticide residues using QuEChERS extraction with dSPE clean-up and LC-MS/MS analysis to ensure its chemical safety and suitability as a feedstock for the recovery of bioactive compounds. To optimize the extraction process, a central composite design coupled with response surface methodology (RSM) was implemented using processing temperature (60–180 °C) and ethanol proportion (0–100%, v/v) as independent variables. Extraction yield, total phenolic content, and in vitro antioxidant activity (evaluated by DPPH free radical scavenging activity, ABTS radical cation scavenging activity, and crocin bleaching inhibition assays) were used as response variables to determine the optimal MAE conditions for maximizing bioactive compound recovery while minimizing solvent consumption and extraction time. Although the experimental work is still ongoing, quadratic polynomial models are expected to be successfully fitted and statistically validated to accurately describe the MAE process and identify the optimal extraction conditions. The conditions predicted by these models will then be experimentally validated by comparing the predicted and experimental responses. To this end, extractions will be performed under the theoretical conditions, and the extraction yield, total phenolic content, and antioxidant activity will be experimentally determined. The extract obtained under these conditions will subsequently be characterized to determine its individual phenolic compounds. Overall, this work is expected to establish an optimized green MAE process for obtaining phenolic-rich extracts from eggplant aerial biomass, supporting their sustainable and safe valorization for future food, pharmaceutical, and cosmetic applications.

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Can traditional meat products become healthier without losing their identity?

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Traditional meat products are an important part of the gastronomic heritage of many regions and are closely associated with local breeds and consumer perceptions of authenticity. However, growing concerns regarding the relationship between diet and chronic diseases have increased pressure on the meat industry to improve the nutritional profile of processed meat products. In particular, the high contents of sodium and saturated fat commonly found in traditional meat products have been identified as major nutritional challenges. Consequently, the development of reformulation strategies capable of improving nutritional quality while preserving the sensory and technological characteristics that define these products has become a priority for both researchers and industry. The Bísaro pig is an autochthonous Portuguese breed widely used in the production of traditional meat products with high cultural value. This project aims to assess the feasibility of producing healthier Bísaro pork products through sodium reduction and animal fat replacement while preserving their characteristic quality attributes.

Two complementary reformulation approaches are currently being investigated. The first consists of the partial replacement of sodium chloride with potassium chloride as a strategy to reduce sodium content while maintaining the technological functions of salt, including flavour enhancement, water retention, texture development and microbiological stability. Different replacement levels are being evaluated to determine their effects on physicochemical characteristics, mineral composition, and sensory acceptance. The second approach focuses on replacing pork fat with hydrogel-based systems designed to reduce saturated fatty acid content while maintaining the structural and technological properties required for traditional meat products. The hydrogel formulations under study are intended to mimic the functional behavior of animal fat and preserve product texture, juiciness,and overall sensory quality[1,2].

The experimental work is being carried out in collaboration with a traditional meat processing company and includes the production of different batches of Butelo, Alheira and Salsichão formulated according to the proposed reformulation strategies.

The impact of sodium reduction and fat replacement is being assessed through a multidisciplinary analytical approach involving physicochemical characterization, mineral determination, texture and colour evaluation, nutritional assessment, and sensory

analysis. Particular attention is being given to balancing nutritional improvement with the preservation of attributes that contribute to product identity and consumer acceptance. Although the project is still ongoing, preliminary observations suggest that partial sodium replacement and hydrogel incorporation can be successfully implemented in traditional Bísaro pork products without major technological constraints during processing. These initial findings support the potential of reformulation strategies as effective tools to improve the nutritional quality of traditional foods while maintaining their characteristic properties. From a food chemistry perspective, this project addresses key challenges associated with the reformulation of traditional meat products and contributes to the development of healthier foods that remain aligned with consumer expectations. The results generated are expected to provide scientific evidence supporting the reduction of sodium and saturated fat in traditional meat products, contributing to public health objectives and responding to current market trends.

Overall, this work explores whether traditional meat products can become healthier without losing the characteristics that define their identity. By combining sodium reduction through partial replacement of sodium chloride with potassium chloride and fat replacement using hydrogel-based systems, the project seeks to establish innovative reformulation strategies capable of preserving authenticity, quality and consumer acceptance while improving the nutritional profile of traditional Bísaro pork products.

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Transforming Grape Stems into Bio-Based Materials

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The winemaking industry generates around 9 million of tons of agricultural waste annually worldwide[1]. In an era focused on sustainability and resource efficiency, transforming this waste into valuable products has become a key priority. Grape stems (GS) are one of the winemaking by-products, accounting for approximately 7 % of the total processed grape weigh[1-3]. Being a lignocellulosic biomass, GS, could be converted into new biomaterials by using biorefinery protocols, thus replacing the petroleum-based materials[1-3].

The present work aimed to evaluate the potential of GS as an ingredient for developing new biomaterials using green technologies. For that, GS was treated by two methods (Figure 1). Method 1 used autohydrolysis (AH) in autoclave (120 ˚C, 60 min, 5 % solid ratio) followed by a Soxhlet extraction of 3 h using ethanol 50%. Method 2 used AH in pressurized reactor (180 ˚C, 30 min, 5 % solid ratio, 150 rpm) followed by Organosolv (180 ˚C, 40 min, 175 rpm, ethanol 55 %). After treatments, insoluble solids were vacuum filtered and washed with 0.3 L of extraction solvent. The remaining solid were mixed with distilled water and homogenized in Ultraturrax at 15000 rpm for 15 min. Glycerol and citric acid were then incorporated in mixture as plasticizer and crosslinking agent, respectively. Biomaterials were obtained after casting at 50 ˚C for 16 h followed by hot-press (temperatures ranging from 130 ˚C to 180 ˚C; and pressures ranging from 1.4 tons to 6 tons).

Results showed that treatments were effective to extract and solubilize hemicelluloses, phenolics and some lignin. The cellulose and lignin were mainly retained in insoluble solids. The formulations tested had brownish colors, flexible behavior and paper, plastic and/or plywood-like behavior.

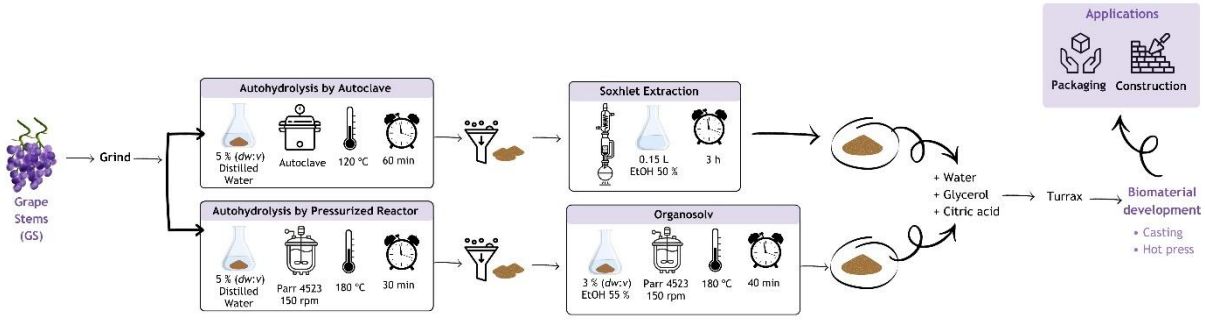


Figure 1 – Resume of the work performed.

In conclusion, this research demonstrated that GS can be effectively upcycled into useful bio-based materials with a lot of potential applications, like food packaging, isolation materials for construction, pallets. etc. Moreover, a more extensive analysis of the resulting products should be performed to validate their usage and optimize their properties. Nevertheless, the results presented here encourages the possibility to transform winemaking wastes into sustainable substitutes for fossil fuel-derived polymers/materials.

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[P.5.7]

The importance of close collaboration between Food Chemistry and the Food Industry in the (re)search for more appealing foods for the rarer cases of food intolerance

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Nowadays, intolerance to gluten or lactose are usual in human populations around the world. Nevertheless, despite food with no gluten or no lactose are widely found in the supermarkets, in restaurants the options are much lower.
For metabolic pathologies related to amino acids intolerances a chance to find out suitable meals in common restaurants are almost impossible to find out. Appeling meals outside home are completely utopic and often young people make incorrect options, to overpass the boring food that they can ingest.
Therefore, the close cooperation between Food Chemistry and Food Industry are the eminent solution to the outcome of new, appellative and healthy products (and meals) that can be suitable for these important and increasing market niches.
An example is tryptophan (Trp) intolerance. Trp is widely found in meat, eggs, dairy products, cereals, beans, soybeans, grains, eggs, tuna, salmon, tofu, chocolate, banana, avocado or nuts. Despite the daily nutritional requirement for L-tryptophan (Trp) is modest (5 mg/kg). Nevertheless, this aromatic amino acid is abundant in diets (either omnivorous or vegetarian) and large amounts of Trp are absorbed. This can lead to several pathological symptoms, being tremors, nausea, and dizziness the less dramatic ones. People that have problems with tryptophan cell transport can easily have other dangerous symptoms like delirium, myoclonus, hyperthermia, and coma (the serotoninergic syndrome).
Hence, it is imperative the conjugation of efforts to attain the requirements of these increasing groups, often forgotten by Food Industry.

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Extraction Technologies for Cannabis Bioactives: A Comprehensive Review

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Cannabis sativa L. is a highly complex plant increasingly recognized within the biorefinery concept for its versatile industrial, medicinal, and nutritional applications[1]. In the context of food chemistry, this plant represents a promising source of bioactive compounds and functional ingredients for food and nutraceutical applications. Utilizing the entire plant offers significant value: flowers yield bioactive cannabinoids and terpenes, seeds provide high-quality oils, and residual biomass holds potential for sustainable bioenergy production or agricultural applications[1]. This review provides a comprehensive summary of the primary bioactive compounds in Cannabis, specifically cannabinoids, terpenes, and flavonoids, focusing on the physicochemical properties that are essential to the extraction process. To evaluate efficient processing methods, conventional extraction techniques (e.g., maceration and Soxhlet) were compared with advanced, green methodologies, including Supercritical Fluid Extraction (SFE), Pressurized Liquid Extraction (PLE), Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE). Advanced technologies, particularly supercritical CO_2 extraction, demonstrate superior selectivity and enhanced preservation of thermolabile, nutritionally valuable compounds[2]. Furthermore, an integrated workflow, detailing pre-extraction processing (drying, grinding, and decarboxylation) and post-extraction purification stages (winterization and distillation), combined with robust analytical characterization using chromatographic (GC, LC, SFC) and spectroscopic (FTIR, NIR, NMR) techniques, is presented (Figure 1). Those methods remain essential for rigorous quality control, standardization, and reproducibility[3]. Ultimately, choosing an extraction strategy must balance yield, safety, and environmental sustainability. Future developments are expected to focus on green solvents, hybrid processes and artificial intelligence tools, as well as the harmonization of analytical methods and regulatory requirements, to ensure the quality, standardization and sustainable development of products based on *Cannabis sativa L.*.

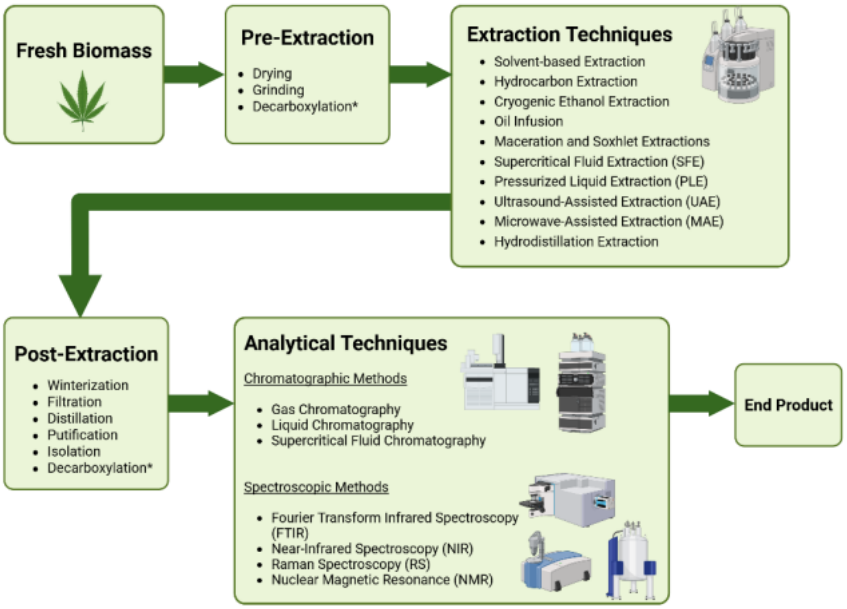


Figure 1 – Overview of the integrated workflow for the extraction and analytical characterization of Cannabis sativa L. bioactive compounds from fresh plant biomass. *Decarboxylation can be strategically applied either pre-extraction or post-extraction, as detailed in the text. Created in https://BioRender.com.

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Funding: Fundação para a Ciência e a Tecnologia through projects UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

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Upcycling Overripe Grapes (*Vitis vinifera L.*) into a Functional Beverage

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Climate change has increasingly impacted wine-producing regions worldwide, particularly in temperate zones, where rising average seasonal temperatures, reduced rainfall, and altered precipitation patterns during the ripening period are changing vineyard conditions and grape maturation[1]. During grape maturation, as climate warms, the sugars and secondary metabolites in grapes gradually become uncoupled during their development, resulting in the phenomenon of overripe grapes. The overripening stage can trigger various physiological, biochemical, structural, and compositional changes in the grape berries, because, as the grape skin cells gradually lose vitality, and consequently the grape berries suffer water loss (dehydration) and weight[2]. Therefore, during harvesting, the overripe grapes have been discarded from the winemaking process because of their high sugar content, modifications in organic acid and phenolic profiles, and lower juice content[1,2]. High sugar concentrations in must cause problems during fermentation, and an unacceptable alcohol content from the point of view of wine quality.1 The redirecting of overripe grapes to produce plant-based fermented beverages is one of the upcycling strategies to reduce post-harvest losses and incorporate nutrients and bioactive compounds. The objective of this study was to investigate the fermentation properties during 24 hours using water kefir grains combined with reconstituted juice from lyophilized overripe grapes (*Vitis vinifera L.*). The physicochemical properties: Total phenolic content (TPC; gallic acid equivalent (GAE)), total flavonoids content (TFC; quercetin equivalent (QE)) and total monomeric anthocyanins (TMA; cyanindin-3-glicoside equivalent (CE)), of reconstituted juice and water kefir (GWK) were determined. As expected, the fermentation increased the concentration of organic acids (lactic and acetic acids) and reduced the pH. The pH was 3.93, while concentrations of lactic and acetic acids were 0.47 g/L and 0.56 g/L, respectively. The results showed that after 24h fermentation process (Figure 1) significantly increase TMA (15.47 mg CE/100mL) compared to initial time (11.24 mg CE/100mL), while TFC displayed no changes (283.30 mg QE/100mL (24h); 272.23 mg QE/100mL (initial time)). However, a slight decrease in TPC (1037.38 mg GAE/100mL) was observed in relation to the initial time (1369.68 mg GAE/100mL).

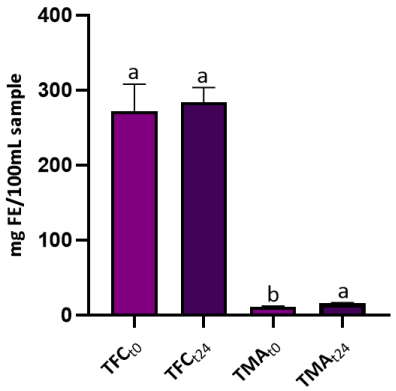


Figure 1 – Total flavonoids content (TFC) and total monomeric anthocyanins (TMA) of reconstituted juice from lyophilized overripe grapes (t0) and grape water kefir (t24). For each analytical determination, different lowercase letters (a, b) in the bars indicate significant differences by unpaired t-test ($p < 0.05$).

In summary, the intake of flavonoids, including the monomeric anthocyanins has evidenced health-promoting effects due to modulation of gut microbiota. Monomeric anthocyanins can go through the portal vein to the liver, and can be directed to the systemic circulation to be taken up by the target organs and tissues, acting in the prevention of diseases associated with oxidative stress. According to the finding, overripe grapes can be redirected to produce functional beverages as a upcycled product for consumers.

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Green recovery of capsaicinoids from chili pepper by-products: A pilot-scale case study

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The chili sauces agrifood industry generates significant amounts of chili pepper by-products that pose a significant environmental challenge, since their direct disposal is highly problematic due to their inherent pungency. However, these agricultural residues can be processed into valuable oleoresins for alternative industrial applications due to their valuable source of bioactive compounds, pigments, and spicy capsaicinoids. Moreover, market analysis indicates a growing sector, evidenced by an increase in new pungent product launches in Western Europe and the United States[1]. This study aimed to screen the impact of industrially available green extraction methodologies, particularly high-pressure processing (HPP) and pulsed electric fields (PEF) on capsaicinoids recovery compared with conventional organic solvent extraction.

The experimental design evaluated Jalapeño by-products. Conventional reflux extraction (1:8 w/v ratio, 96 % ethanol) was conducted as a benchmark under different time and temperature conditions (1 and 5 h; 50 and 80 °C). For high-pressure technologies, HPP with an aqueous pre-treatment stage (1:20 w/v ratio, 400 MPa, 6 min) was employed, followed by an ethanolic high-pressure-assisted extraction (HPE, 1:8 w/v ratio, 600 MPa, 6 min, 96% ethanol) across under different temperatures (room temperature, 50 and 80 °C). Additionally, PEF pre-treatment was carried out with an electric field intensity of 2 kV/cm, a medium temperature of 30 °C, and a processing time of 2500 µs, followed by ethanolic extraction (1:8 w/v ratio). This approach aimed to evaluate the role of PEF-induced electroporation in enhancing mass transfer during capsaicinoid recovery. Capsaicinoids concentration was monitored via Scoville Heat Units (SHU) through HPLC quantification[2], alongside the total oleoresin laboratory mass yield (%).

The results did not reveal a significant impact of temperature and time on capsaicinoids extraction by reflux, being 1 h and 50 °C the balanced operational conditions. Under these conditions, reflux extraction yields 16% of oleoresin with 26 kSHU. Regarding high pressure, room temperature allows for higher levels of capsaicinoids. This could be particularly interesting for oleoresins rich in thermosensitive compounds. Overall, the incorporation of HPP as a by-product pre-treatment in reflux extraction resulted in less spicy oleoresins, 21.5 kSHU and 22 kSHU under control (pre-treatment with water without HPP following reflux). Similarly, a decrease in oleoresin spiciness was observed upon combining HPP with HPE, resulting in oleoresins with 12 kSHU alongside ~ 1 % of oleoresin yield. The respective control (HPP with HPE conditions without high pressure) resulted in 15 kSHU oleoresin. Although HPP pre-treatment did not significantly enhance capsaicinoid extraction, it may help to remove water-soluble impurities that typically precipitate during the oleoresin concentration process, explaining the lower yields obtained under these conditions. Furthermore, PEF-assisted extraction enhanced capsaicinoid recovery compared with the control, achieving 80 kSHU under the applied conditions. Prolonged conventional heating (80 °C, 5 h) trigger thermal degradation, drastically reducing capsaicinoid recovery. This study provides a comprehensive preliminary understanding of the possible outcomes from these industrially viable technologies for spicy capsaicinoids extraction, establishing the foundational parameters required to implement an optimized pilot-scale extractive process successfully.

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Effect of moisture content on ergosterol extraction from *Agaricus bisporus* (J.E.Lange) Imbach

Cardoso, R. V. C.[1,*]; Lopes, Y.[1]; Delgado, D.[2]; Henriques, A. C.[3]; Ribeiro, S.[4], Fernandes, L.[1,5]; Martins, F.[1,5]; Gonçalves, A.[1,5]; Fernandes, Â.[1]; Barros, L.[1]

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Ergosterol is the main precursor of vitamin D_2 in mushrooms; therefore, its preservation during processing is a key factor influencing the efficiency of subsequent photoconversion and the production of high-value bioactive ingredients[1]. This study aimed to evaluate the effect of different residual moisture contents (3.0%, 3.5%, 5.0%, and 6.0%) in Agaricus bisporus (J.E.Lange) Imbach, aiming to identify the most suitable processing conditions for obtaining ergosterol-rich extracts for subsequent vitamin D_2 production. A. bisporus mushrooms, supplied by Mogaricus, Lda., were dried in a dehydrator until reaching target moisture contents of 3.0%, 3.5%, 5.0%, and 6.0%. The dried samples were then ground and subjected to heat-assisted maceration using ethanol as the extraction solvent (81.6 °C, 150 min)[2]. Ergosterol was identified and quantified by high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). Samples dried to 3.5% residual moisture tended to exhibit higher ergosterol recovery (around 58 mg/g extract), indicating that small variations in the drying process can influence extraction performance. These preliminary results confirm that residual moisture is an important processing parameter affecting ergosterol recovery from A. bisporus. The findings highlight the need to optimise the drying step as part of the overall processing strategy, ensuring improved extraction efficiency and providing suitable raw material for the subsequent photoconversion of ergosterol into vitamin D_2. The knowledge generated in this activity contributes to optimising the production process and supports the development of high-value natural ingredients.

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Evaluation of drying conditions prior to ultrasound-assisted ergosterol extraction from mushrooms

Cardoso, R. V. C.[1,*]; Lopes, Y.[1]; Delgado, D.[2]; Henriques, A. C.[3]; Ribeiro, S.[4], Fernandes, L.[1,5]; Martins, F.[1,5]; Gonçalves, A.[1,5]; Fernandes, Â.[1]; Barros, L.[1]

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Ergosterol is the main precursor of vitamin D_2 in mushrooms, making efficient extraction a crucial step in developing natural vitamin D_2-rich ingredients[1]. Optimizing the drying process is therefore essential to maximize ergosterol recovery and improve the efficiency of subsequent processing steps. To evaluate the influence of the final moisture content of *Agaricus bisporus* (J.E.Lange) Imbach on ergosterol extraction efficiency using ultrasound-assisted extraction (UAE), and to identify the most suitable drying conditions for further optimization studies. A. bisporus mushrooms, supplied by Mogaricus, Lda., were dried to final moisture contents of 3.0%, 3.5%, 5.0%, and 6.0%. After drying, the samples were ground and subjected to UAE using ethanol as the extraction solvent (375 W, 15 min)[2].

Ergosterol was identified and quantified by high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). Based on these results, the most promising drying conditions will be selected to evaluate the influence of grinding particle size (mesh) on ergosterol extraction efficiency. Ergosterol recovery varied among the drying conditions evaluated, suggesting that final moisture content influences extraction performance. Samples dried to 6.0% residual moisture tended to show the highest average ergosterol content (around 78 mg/g of extract), whereas the lowest average recovery was observed for samples dried to 3.0% (around 30 mg/g of extract). These findings will support the selection of the most suitable samples for subsequent studies investigating the effect of grinding particle size on ergosterol extraction. In the longer term, this work will help optimize the overall process for obtaining ergosterol-rich extracts and natural vitamin D_2-derived ingredients.

Acknowledgements: This work was supported by the European Union through the NORTE 2030 Regional Programme under the project D2INNOV – Nutritional and Nutraceutical Fortification with Vitamin D Derived from Mushrooms (Project No. 18407; Code: NORTE2030-FEDER-01474100), within the MPr-2023-8 – SIID – Business R&D – Co-promotion Operations (Low-Density Territories). The authors also acknowledge national funding from the Foundation for Science and Technology (FCT, Portugal) through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2025 (DOI:10.54499/UIDB/00690/2025) and UIDP/00690/2025 (DOI: 10.54499/UIDP/00690/2025); and SusTEC, LA/P/0007/2020 (DOI:10.54499/LA/P/0007/2020).
Funding: The authors acknowledge national funding from the Foundation for Science and Technology (FCT, Portugal), under the institutional scientific employment stimulus programme contract for L. Barros (CEEC-INST, DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002) and Â. Fernandes (FCT-TENURE: 2023.11031.TENURE.014). The authors also acknowledge funding for the contract of Rossana V.C. Cardoso through the D2INNOV project.

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Integrated Valorisation of Watermelon By-products through Lactic Acid Fermentation and Sustainable Food Prototypes

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Watermelon (*Citrullus lanatus*) is widely consumed due to its refreshing sensory properties, high water content and nutritional value. Composed of approximately 93% water, this fruit is highly perishable but also represents a promising matrix for fermentation-based applications. Structurally, watermelon comprises relevant fractions of juice, rind and residual pomace, which are commonly discarded during consumption and processing. These by-products, particularly the rind and residual pulp, are valuable sources of fibre, minerals and bioactive compounds, highlighting their potential for food valorisation[1,2]. The recovery and transformation of these fractions can contribute to reducing food waste, promoting circular economy strategies and generating innovative food products with added nutritional and functional value.

The present study explores the potential of two watermelon matrices, pulp and rind, as substrates for lactic acid fermentation and the subsequent development of value-added food products. Both matrices were fermented under comparable conditions using the same lactic acid bacterium, Lactiplantibacillus plantarum BAL 97, at approximately 10^8 CFU/mL, for 10 days at room temperature[3]. Pasteurised, non-inoculated samples were used as controls. The fermentation process was monitored through microbiological and physicochemical analyses, including lactic acid bacteria counts, total aerobic microorganisms, yeasts and moulds, pH, titratable acidity, soluble solids and colour parameters. For the rind matrix, total phenolic content and antioxidant capacity were also evaluated. In parallel, watermelon pulp and rind were valorised through the development of different food prototypes, whose nutritional value was assessed to evaluate their potential as sustainable food products. The pulp was successfully used to produce a fermented watermelon-based beverage, while the rind was incorporated into frozen dessert prototypes, including a sorbet and a creamy ice cream.

Both watermelon matrices supported lactic fermentation, although their responses differed according to composition and structure. In the pulp, fermentation caused a marked pH decrease, increased titratable acidity, and reduced soluble solids, indicating organic acid production by L. plantarum and the utilisation of fermentable sugars. These changes confirm active lactic fermentation and support the suitability of watermelon pulp for fermented beverage development. However, the spontaneous acidification and microbial instability observed in the control highlight the need for controlled fermentation and appropriate thermal treatment to ensure safety, stability, and reproducibility.

Similar acidification was observed in the fermented rind, with decreasing pH, increasing titratable acidity, and reduced soluble solids, confirming sugar consumption by lactic acid bacteria. Fermentation also altered texture and appearance, likely due to structural changes in the plant tissue. Notably, it improved the rind's functional profile by increasing total phenolic content and antioxidant capacity, suggesting that lactic fermentation may release bound phenolic compounds and enhance the bioactive potential of watermelon rind. The product development stage demonstrated the feasibility of valorising both watermelon pulp and rind in food formulations. The nutritional evaluation showed that watermelon rind contributed to fibre enrichment, whereas the pulp provided natural sugars, colour, and characteristic flavour. Differences between the frozen dessert formulations were mainly related to their ingredient composition: the sorbet presented lower energy and fat contents, while the creamy ice cream showed higher lipid content due to the incorporation of dairy ingredients, which likely contributed to improved texture and mouthfeel. Overall, the use of both watermelon fractions enabled the development of products with distinct nutritional and sensory profiles, supporting their potential application in innovative and sustainable food products.

These results highlight the potential of watermelon pulp and rind as suitable substrates for lactic acid fermentation and as ingredients for sustainable food innovation. Controlled fermentation with L. plantarum may contribute not only to biopreservation but also to the enhancement of the functional value of watermelon by-products. Further studies should optimise fermentation conditions, improve microbial stability, assess shelf-life and perform extended sensory evaluation to support future scale-up and industrial application.

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Major Bioactive Phenolics from Sweet Cherry By-Products: Insights into Antioxidant and P-glycoprotein Modulatory Activity

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Sweet cherry (*Prunus avium L.*) production and processing generates several amounts of by-products that remain largely unexploited despite their richness in bioactive compounds with potential biological activity[1]. Recent studies have focused on the phytochemical composition and biological activities of cherry stems, leaves and flowers, identifying major phenolic compounds in these matrices, including hydroxycinnamic acids (neochlorogenic, caffeic, and ferulic acids), flavonols (kaempferol and kaempferol 3-O-rutinoside), and flavanones (naringenin 7-O-glucoside and sakuranetin)[2].

In this study we investigate the antioxidant activity and P-glycoprotein (P-gp) modulatory capacity of these major phenolic compounds and of hydroethanolic extracts and infusions prepared from sweet cherry stems, leaves and flowers. Antioxidant activity was assessed through nitric oxide (•NO) and superoxide (O_2•$^-$) scavenging assays, revealing distinct profiles among compounds: neochlorogenic acid and naringenin 7-O-glucoside showed the strongest •NO inhibition, whereas kaempferol exhibited the highest activity against O_2•$^-$.

Extracts and isolated compounds were further evaluated in MDCK-MDR1 cells using rhodamine 123 (Rh123) accumulation to assess P-gp inhibition. The stems hydroethanolic extract produced the most pronounced increase in intracellular fluorescence, indicating partial inhibition of P-gp. Among isolated compounds, kaempferol and its rutinoside derivative displayed concentration-dependent effects consistent with extract activity, while naringenin 7-O-glucoside and sakuranetin exhibited biphasic responses suggestive of complex transporter interactions.

Overall, these results demonstrate that sweet cherry stems, leaves and flowers contain major phenolic constituents with dual antioxidant and P-gp-modulating properties. Their combined activity highlights the potential of these by-products as valuable sources of bioactive molecules for nutraceutical development and for strategies aimed at mitigating.

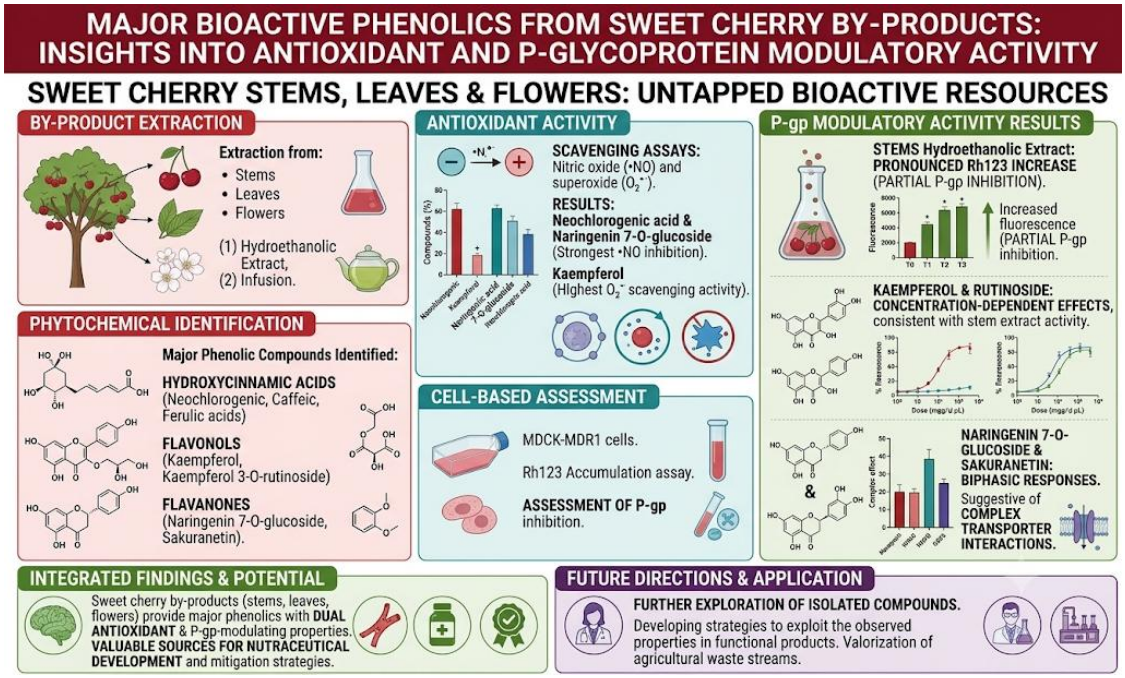


Figure 1. Valuation of sweet cherry (*Prunus avium L.*) by-products: An integrated framework mapping phytochemical composition, antioxidant mechanisms, and P-glycoprotein modulatory activity.

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[P.5.15]

Circular Valorization of Fruit Peels: Exploring the Potential of Apple, Orange and Banana

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The generation of organic waste remains a major environmental and economic challenge for the food industry. Among the various by-products produced during fruit processing, peels account for a significant fraction and are often underutilized despite their valuable chemical composition. In this context, the recovery and valorization of biological resources aligns closely with the principles of the circular economy, which promotes the efficient use of resources, waste minimization, and the creation of added value (e.g. recovery of compounds of interest). These objectives are also consistent with the ambitions of the European Green Deal and the transition towards more sustainable production systems. The present study aimed to evaluate the valorization potential of Malus domestica Borkh., cv. Gala; average fruit mass: 110 g), Bahia orange (Citrus sinensis (L.) Osbeck; average fruit mass: 340 g), and commercially available banana (Musa spp.; average fruit mass: 356 g) peels through peel yield, main soluble sugars (by High-Performance Liquid Chromatography), and Ca and K content (X-ray fluorescence). The peels were manually separated from the edible portions, and peel yield was calculated as the ratio between dry and fresh mass after dehydration, and expressed as a percentage. The remaining analysis of sugars and minerals were performed after milling of the dry fruit peels. The results revealed marked differences, with apples, orange, and banana peels showing yields of c.a. 21%, 25%, and 10%, respectively, demonstrating that the amount of potentially available by-product varies considerably among fruits. Apple peel exhibited the highest total soluble sugar content, with fructose (405 mg g^{-1} DW), glucose (218 mg g^{-1} DW) and sucrose (92 mg g^{-1} DW) as the predominant sugars. Banana peel contained 10 mg g^{-1} DW raffinose, 144 mg g^{-1} DW sucrose, 71 mg g^{-1} DW glucose and 98 mg g^{-1} DW fructose. Orange peel exhibited 12 mg g^{-1} DW raffinose, 65 mg g^{-1} DW sucrose, 129 mg g^{-1} DW glucose, and 136 mg g^{-1} DW fructose. Elemental characterization (% DW) also revealed distinct profiles. Orange peel presented the highest Ca content (3.2%), whereas banana peel was characterized by a high K content (5.9%). Apple peel contained 0.7% Ca and 0.4% K. These results highlight the distinct compositional profiles of the three matrices, suggesting that each fruit may require a specific valorization strategy to fully exploit its potential. Apple peel therefore stood out for its high soluble sugar content, orange peel for its high biomass yield after dehydration and elevated Ca content, and banana peel for its high K content. The direct use of milled fruit peels, the recovery of soluble compounds, and the exploitation of mineral-rich fractions represent complementary valorization strategies for the development of value-added ingredients. The approach adopted reinforces the potential of fruit peels as resources for the circular bioeconomy, highlighting that more efficient valorization may result from the development of differentiated applications based on the specific profile of each by-product.

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[P.5.16]

Water mineralization modulates phenolic and volatile profile in olive leaf infusions: a chemical basis for product valorization

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Olive leaves are a high-volume by-product generated in large quantities by the olive oil industry, and their valorization into functional infusion products represents an attractive strategy within circular bioeconomy approaches. These leaves are particularly rich in phenolic compounds, notably oleuropein (OL) and its hydrolysis product hydroxytyrosol (HT), both recognized for their antioxidant and health-promoting properties, and in aroma active volatile compounds that contribute to the sensory identity of the infusion[1]. Although extraction conditions such as temperature and time are widely studied, the role of water composition, and particularly its mineral content, remains largely overlooked as a variable capable of shaping the final chemical profile of olive leaf infusions. This study aimed to evaluate how water mineralization influences the phenolic transformation, volatile composition, and antioxidant-related potential of olive leaf infusions, providing a mechanistic basis for tailoring product composition through a simple, formulation-based approach. Olive leaf infusions were prepared using waters with distinct mineralization levels, ranging from 200 to 1033 mg/L total dissolved solids. Volatile compounds were analyzed by GC-MS, while phenolic compounds were quantified by HPLC-UV-ED. GC-MS analysis identified nonanal as the predominant volatile compound across the samples. Regarding phenolic composition, HPLC-UV-ED quantification revealed a clear mineralization-driven conversion of OL into HT since infusions prepared with highly mineralized water exhibited significantly lower OL and correspondingly higher HT concentrations, consistent with mineralization-promoted hydrolytic cleavage of OL, while low-mineralization water preserved OL largely intact. Importantly, electrochemical detection showed that this conversion was accompanied by a net decrease in electrochemically active phenolics, and consequently in antioxidant-related activity, as mineralization increased. Taken together, these results establish water mineralization as a controllable extrinsic factor governing phenolic transformation, volatile release, and antioxidant potential in olive leaf infusions. This mineralization-driven modulation reinforces the potential of olive leaves as a versatile, tailorable ingredient for functional food applications and supports the development of economically and environmentally relevant valorization pathways for this abundant agricultural residue.

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Integrated utilization of phenolic-rich extract and fiber-rich residue from discarded plum for the development of clean-label fruit preserves

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The sustainable valorization of fruit by-products requires strategies capable of maximizing the use of all biomass fractions while maintaining food quality and functionality. Discarded plums represent an important source of phenolic compounds and dietary fiber, enabling the simultaneous recovery of extracts rich in antioxidants and the utilization of the remaining residue rich in fibers as food ingredients. In this context, this study investigated the integrated application of both fractions in the development of clean-label fruit preserves and evaluated their nutritional composition, antioxidant potential, and physicochemical stability during storage. An extract rich in phenolic compounds from plum was previously obtained by microwave-assisted extraction (MAE), while the corresponding post-extraction residue was recovered for food application. Four fruit spread formulations were developed: a control without preservatives, a formulation containing potassium sorbate, a formulation supplemented with 1% (w/w) of the optimized plum extract, and a formulation containing 1% (w/w) of the optimized extract combined with 2% (w/w) of the corresponding post-extraction residue. Nutritional composition, including moisture, protein, fat, ash, total carbohydrates, dietary fiber and sugars, was determined according to AOAC methods[1]. In addition, the formulations were subjected to a 42-day storage study, during which pH, water activity, color, total phenolic content, and antioxidant activity were monitored to assess product stability.
The integrated experimental design enabled the simultaneous evaluation of the technological performance and nutritional impact of incorporating both the extract rich in phenolic compounds and the residue with fiber content into fruit preserves. The comparison between conventional preservation, phenolic enrichment, and the combined utilization of extract and residue provides valuable information regarding the contribution of each biomass fraction to product composition and stability throughout storage. Collectively, this study demonstrates the feasibility of integrating both the phenolic-rich extract and the corresponding fiber-rich residue into fruit preserves, providing a comprehensive basis for evaluating their contribution to nutritional composition and storage stability while promoting a more sustainable utilization of discarded plum biomass.

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[P.5.18]

Circular economy in the juice industry: Valorization of apple by-products for the bioactive enrichment of multifruit beverages and its impact in phenolic and vitamin profiles during storage

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The circular economy in the juice industry focuses on the valorization of co-products to reduce waste and promote environmental sustainability through their reintegration into the value chain. Apple pulp, derived from juice processing, stands out as a co-product with high valorization potential due to its richness in bioactive compounds, including polyphenols and vitamins. Thus, its incorporation into the development of novel multifruit beverages emerges as a promising strategy to enrich the nutritional profile of these products. However, the stability of these bioactive compounds is highly sensitive to technological factors and extended storage periods[1, 2]. Therefore, monitoring the evolution of these profiles over time is essential to guarantee final product quality and ensure the functional benefits of this incorporation.

Three pulpy multifruit juices were evaluated over a 6-month storage period, with sampling points at day 0 (initial), after accelerated aging in an oven at 40 °C (7 and 14 days) and during ambient storage at 20 °C (3 and 6 months). For polyphenol extraction, 5 mL of each juice was diluted (1:1, v/v) with water and centrifuged. The supernatant was purified using C18 solid-phase extraction cartridges, previously conditioned with 20 mL of methanol and washed with 30 mL of water. Interferences from water-soluble compounds were removed with 50 mL of water. The retained polyphenols were then sequentially eluted with 5 mL of methanol, 5 mL of methanol/acetone (1:1, v/v), and 5 mL of acidified methanol/acetone (1:1, v/v with HCl). The purified extracts were concentrated under vacuum using a rotary evaporator at 38 °C to approximately 1 mL and reconstituted to a final volume of 2 mL.

For B-complex vitamins, centrifuged juice supernatants were filtered (0.22 µm) and analyzed by LC-MS/MS using an HPLC Vanquish coupled to a TSQ Altis Plus triple quadrupole mass spectrometer. Separation was achieved on an Acclaim™ Polar Advantage II column (3 µm, 3×150 mm) at 40 °C with 0.1% formic acid in water (A) and methanol (B) under a multi-step gradient (0.350 mL/min, 10 µL injection). Vitamins B3 (nicotinamide), B5 (calcium-d-pantothenate) and B6 (pyridoxine hydrochloride) were quantified in positive ESI mode via Selected Reaction Monitoring (SRM) using optimized transitions and collision energies. Quantification was performed via matrix-matched standard curves in GraphPad Prism 10.

Polyphenol profiling (20 µL injection) was performed via HPLC Elite LaChrom using an Agilent Poroshell 120 EC-C18 column (2.7 µm, 4.6×250 mm) with 0.5% formic acid in water (A) and acetonitrile (B) under a multi-step gradient. LC-MS in negative ESI mode ([M-H]-) identified compounds by monitoring (m/z) and fragmentation patterns compared with literature. Polyphenols were classified into hydroxycinnamic and hydroxybenzoic acids, xanthones, flavonols, dihydrochalcones and catechins. Quantification used calibration curves of representative standards under identical conditions: (+)-catechin, quercetin-3-O-glucoside, chlorogenic acid, phloridzin, gallic acid and mangiferin.

Accelerated aging at 40 °C induced a progressive degradation of phenolic compounds over 14 days. Conversely, ambient storage at 20 °C led to a pronounced increase in total polyphenols at 6 months across all formulations, driven primarily by the release of the catechin fraction. Regarding the B-complex vitamins, distinct degradation kinetics were observed; vitamin B5 proved to be highly unstable, showing a sharp and continuous decline under all conditions. Vitamin B3 remained relatively stable during accelerated aging but suffered significant degradation over 6 months of ambient storage. Interestingly, vitamin B6 content exhibited a transient increase at 7 days/40 °C—likely due to the thermal release of matrix-bound forms—before gradually declining by the end of the 6-month period.

These findings indicate that while long-term storage promotes the beneficial release of bound polyphenols from the apple pulp into the soluble fraction, it simultaneously compromises the stability of water-soluble vitamins, particularly B5 and B3. Therefore, the valorization of apple pulp is a highly viable and sustainable strategy for the phenolic enrichment of multifruit beverages with enriched and long-term stable bioactive profiles.

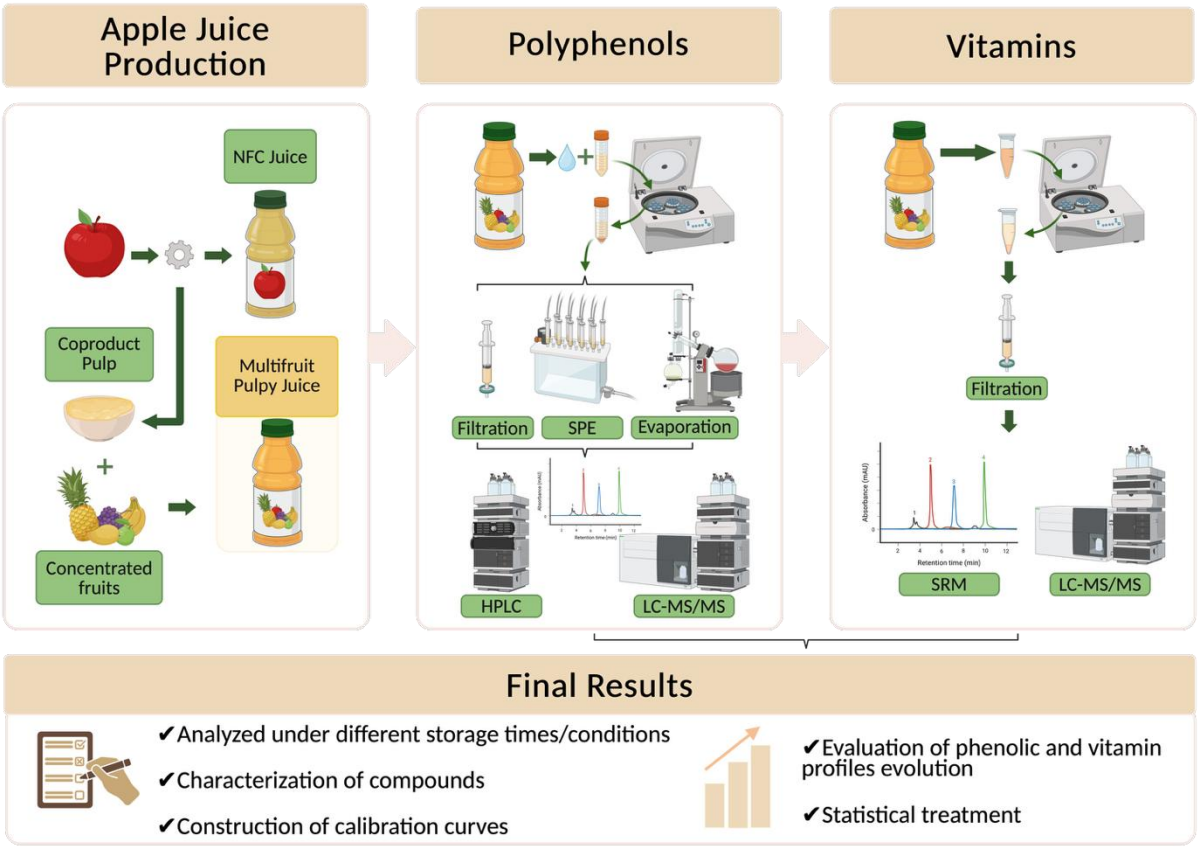


Figure 1 - Graphical abstract of the experimental procedures.

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Valorization of agro-industrial byproducts: apple peel and tomato pomace as sources of bioactive compounds

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In the framework of the circular economy, the valorisation of agro-industrial by-products and residues into valuable resources is crucial for advancing environmental and economic sustainability. The food processing sector produces large quantities of underutilized waste that presents disposal challenges, although it has significant potential as a reservoir for bioactive compounds. This study investigates the bioactive properties of extracts obtained from two common food industry residues: apple peel and tomato manufacturing residue. The extracts were achieved via extraction with acidified ethanol, concentrated using a rotary evaporator, and subsequently freeze-dried. The antioxidant profile of the extracts was evaluated through total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging assays, while the individual phenolic fingerprint was analysed by LC-MS. Moreover, the antimicrobial efficacy against key foodborne pathogens (Staphylococcus aureus and Escherichia coli) was assessed by determining the minimum inhibitory concentration (MIC). The extraction process yielded approximately 48.4 % (w/w) for the apple peel extract (APE) and 7.9 % (w/w) for the tomato manufacturing residue extract (TMRE). Analytical assays revealed that TMRE contained a slightly higher phenolic content, with 14.58 ± 0.51 mg gallic acid equivalent per gram (GAE/g) but lower flavonoid levels, 4.86 ± 0.52 mg catechin equivalents/g (CatE/g), compared to APE (TPC: 13.79 ± 1.06 mg GAE/g; TFC: 9.12 ± 0.52 mg CatE/g). LC-MS analysis successfully identified several phenolic compounds, including epicatechin, quercitrin, and caffeic acid in APE; phloretin, naringenin, and quercetin in TMRE; as well as rutin and chlorogenic acid in both extracts. Regarding antioxidant performance, APE exhibited stronger DPPH scavenging activity, with a lower IC50 value (1.75 mg/mL) than TMRE (3.38 mg/mL). Both matrices showed antimicrobial properties, with higher potential against the Gram-positive S. aureus than the Gram-negative E. coli. These findings establish a basis for subsequent research focusing on integrating these bioactive extracts into bio-based polymeric matrices to develop functional and sustainable active food packaging products.

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From by-product to functional ingredient: Oyster mushroom (*Pleurotus ostreatus*) stem flour as a partial meat replacer in beef burgers

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The oyster mushroom (*Pleurotus ostreatus*) is a widely consumed edible fungus, valued for its sensory attributes and for a nutritional profile associated with hypoglycaemic, hypolipidemic, antioxidant, antibacterial, anti-inflammatory and immunomodulatory effects [1,2]. These properties, linked to its content in polysaccharides, proteins, phenolic compounds, amino acids, vitamins and fatty acids, place *P. ostreatus* within the category of functional foods. However, industrial processing generates substantial amounts of by-products, particularly the stems, that are often discarded despite their bioactive potential, making them a promising resource within circular economy strategies[3].

This work investigated the use of *P. ostreatus* stem flour as a partial meat replacer in reformulated beef burgers. Oyster mushrooms were dehydrated at 50 °C for 8 hours using a dehydrator prior to processing. Beef was ground in a meat grinder, then combined with oyster mushroom flour and 1% salt in a Bimby (TM6) and mixed for 2 minutes. Four formulations were prepared: a control (100% beef) and three experimental formulations in which 5%, 10%, and 20% (w/w) of the beef was replaced with mushroom stem flour (see Figure 1). Both the oyster mushroom flour and the final formulations were characterised in terms of proximate composition, moisture, protein, fat and ash contents, water activity and colour (CIELab). Texture analysis was performed for both raw and cooked hamburgers, evaluating hardness, cohesiveness, springiness, gumminess, and chewiness. Their technological performance, namely cooking yield and diameter reduction, was assessed in burguer formulations. A sensory evaluation, based on a quantitative descriptive analysis, focused on visual and olfactory attributes was carried out on raw and cooked burgers. The assessment considered colour, surface brightness, uniformity, visibility of mushroom particles, degree of browning after cooking, meat-like aroma, mushroom aroma, and off-odours. The results highlight the potential of mushroom by-products as functional ingredients for burger reformulation, supporting product innovation, waste minimisation and alignment with the United Nations Sustainable Development Goal 12 (Responsible Consumption and Production).



Figure 1 - Burgers formulated with different levels of *P. ostreatus* stem flour: (a) control (0%); (b) 5%; (c) 10%; and (d) 20%.

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From Winery By-Products to Cheese Preservation: Vinified Red and White Grape Skin Flours as Natural Surface Biopreservatives for Ripened Sheep Cheese

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Driven by circular economy and clean-label principles, the food industry has increasingly focused on the valorisation of agro-industrial by-products[1], such as grape pomace. Although grape seeds can be valorised through the extraction of oil and other high-value compounds, the remaining fractions, particularly grape skins, still have limited industrial applications. Grape skins account for approximately 50% of the total grape pomace mass, while the remaining fraction consists mainly of seeds and stems.

This study evaluated the surface application of grape skin flours obtained from the red (Touriga Nacional) and white (Arinto) grape varieties, previously milled and sieved to a particle size below 250 µm, on Serpa PDO sheep's milk cheese to investigate their antimicrobial and antifungal potential. The flours were applied at two different stages of ripening, on days 8 and 16. These application times were selected to coincide with key stages of the traditional Serpa PDO cheesemaking process. Day 8 corresponds to the stage at which natamycin is conventionally applied in commercial production, whereas day 16 coincides with the change in ripening temperature and relative humidity. Following treatment, all cheeses continued the ripening process under the conditions established in the Serpa PDO product specification.

After 30 days of ripening, corresponding to the minimum maturation period required for Serpa PDO commercialization, surface samples were collected for the enumeration of moulds, yeasts, mesophilic microorganisms, and Pseudomonas spp. In addition, physicochemical analyses were performed to evaluate the effects of the treatments and to verify that the overall quality of the final product was not adversely affected.

The microbiological results are presented in Figure 1.

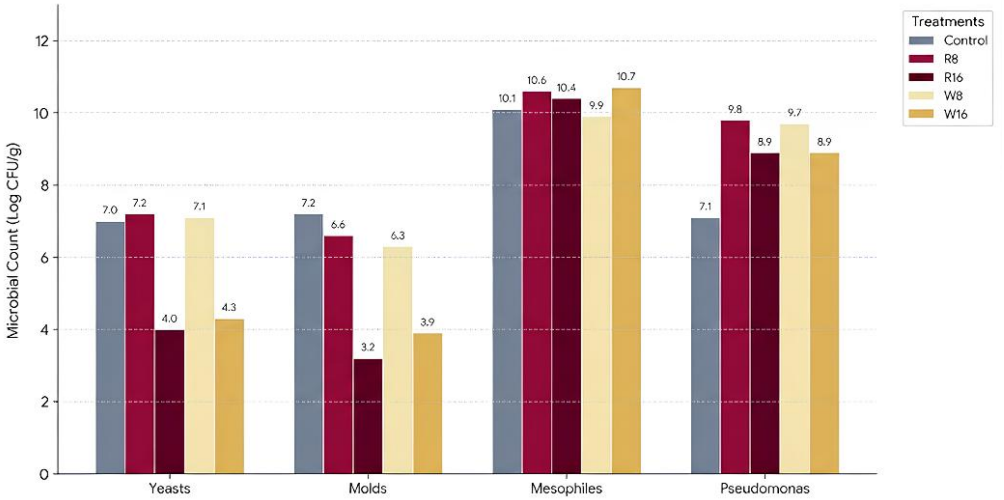


Figure 1 - Comparative evaluation of microbial populations on the surface of Serpa PDO cheeses after 30 days of ripening. Bars represent microbial counts (log CFU/g) for the untreated control and cheeses treated with red (R) and white (W) grape skin flours. Numerical suffixes indicate the day of treatment application (8 or 16 days of ripening).

Cheeses treated on day 16 (R16 and W16) exhibited a marked reduction in yeast and mould counts compared with the control and the day 8 treatments, whereas mesophilic populations remained statistically similar across all experimental groups. In contrast, Pseudomonas spp. counts increased in all treated samples, particularly in R8 and W8, suggesting that the treatments were considerably more effective against fungal populations than against Pseudomonas spp. Overall, application of the grape skin flours on day 16 provided the greatest microbiological control during.

These preliminary results demonstrate the potential of vinified grape skin flours as natural surface antimicrobial agents for Serpa PDO sheep cheese, particularly when applied at later stages of ripening. Ongoing research will focus on long-term storage trials to evaluate the persistence of the antimicrobial effect and the impact of the treatments on the physicochemical and sensory stability of the cheeses throughout their commercial shelf life.

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Microwave Pasteurization of Cloudy Apple Juice: Chemical Characterization and Dietary Fiber Enrichment

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Apple juice is widely consumed worldwide due to high shelf life, manufacturing capabilities, and good nutritional value. However, despite its popularity, clear juice is not considered a valuable source of bioactive substances when compared to its cloudy form, since cloudy apple juice retains natural colloidal suspensions, composed of pectins, proteins, and free amino acids.

Regarding the pasteurization of fruit juices, conventional methods (thermal processing) report challenges such as high energy consumption, impact on the sensory and nutritional properties, prolonged processing times and high operating costs. Growing public health concerns coupled with the need to adopt more sustainable and efficient practices in the food industry have stadriven demand for innovative technologies. Microwave processing offers a promising alternative for juice stabilization, combining shorter processing times with high microbial inactivation efficiency, allowing for better preservation of quality, thereby retaining a greater proportion of the nutritional and sensory properties of apple juices 1. The increase in soluble dietary fiber (SDF) compared to insoluble dietary fiber (IDF) adds functional properties to the final product, such as prebiotic effect. Fruit juices provide SDF, contributing significantly to the daily intake of this type of dietary fiber. Previous studies have reported that pasteurization of fruit pulps can promote an increase in soluble dietary fiber (SDF), enhancing their functional properties 2. Within this context, the PROFITWAVE project aims to develop a continuous microwave pasteurizer, combining numerical simulation and experimental validation to design, build, and validate laboratory-scale prototypes, while minimizing browning, preserving sensory attributes, and increasing SDF content. The cloudy apple juice was analyzed at three stages of the production line: after pressing (AP), before conventional pasteurization (PP), and after conventional pasteurization (P); the samples were collected at Indumape – Industrialização de Fruta, S.A. The samples were initially characterized in terms of their physicochemical properties, namely soluble solids content, density, conductivity, acidity, and the contents of ascorbic acid, malic acid, phenolic compounds, and total dietary fiber (SDF and IDF). AP presented a dietary fiber content of 0.44 g/100 g juice, comprising 0.29 g SDF/100 g and 0.15 g IDF/100 g, and was therefore selected for the heating experiments. Preliminary heating tests were performed using a domestic microwave oven equipped with an 800 W magnetron, while COMSOL Multiphysics® was employed to simulate the heating process 3. Different combinations of temperature (75–90 °C) and processing time (140–210 s) were evaluated. The heating conditions were investigated to establish suitable operating parameters for subsequent transfer to the laboratory-scale microwave pasteurizer currently under development. Microwave heating promoted the formation of visible aggregates within the juice accompanied by a 45% Relative Browning Index after heating. Dietary fiber analysis further showed a redistribution of the fiber fractions with SDF decreasing from 0.29 to 0.22 g /100 g of juice and IDF increasing from 0.15 to 0.22 g/100 g juice, indicating that further optimization of processing conditions is required. The addition of 0.5 g/L ascorbic acid prior to microwave heating, combined with juice homogenization every 30 s, improved temperature distribution and produced a homogeneous suspension without phase separation or aggregate formation, providing a basis for further optimization aimed at preserving dietary fiber composition. Overall, these preliminary results demonstrate the importance of optimizing microwave processing conditions to preserve the physicochemical stability of cloudy apple juice while supporting the development of an industrially scalable microwave pasteurization process.

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From olive pomace to value-added biocompounds: an integrated green extraction and molecular screening approach

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Olive pomace is a major by-product of the olive oil industry, whose residual lipid, phenolic and lignocellulosic fractions constitute a potential source of bioactive compounds[1]. Its valorisation through sustainable extraction technologies may support the development of new high-value applications while reducing the environmental impact associated with this agro-industrial residue. This work investigated an integrated approach combining supercritical fluid extraction (SFE), preliminary chromatographic characterization, and molecular dynamics simulations, with particular emphasis on the potential application of recovered biocompounds with antimicrobial activity against the phytopathogenic bacterium Xylella fastidiosa.

Supercritical CO_2 extraction was performed using wet and dried olive pomace, with pure and CO_2 + water or ethanol as modifiers, and different operation conditions. Extraction kinetics and global yields demonstrated that process performance was strongly influenced by matrix moisture and modifier polarity. The highest global extraction yield, 73.36%, was obtained from dried olive pomace using ethanol as co-solvent, highlighting the importance of matrix pretreatment and solvent selection for enhancing compound recovery.

The extracts were qualitatively characterized by thin-layer chromatography (TLC) using three orthogonal mobile phases targeting different compounds, and plates were visualized under UV light at 365 nm and with derivatization through selective reagents, including $FeCl_3$, NEU and vanillin/sulfuric acid. The resulting chromatographic fingerprints (Figure 1, left and center) revealed differences among extraction conditions and indicated the coexistence of phenolic and lipophilic fractions. Among the available biocompounds in the extracts, hydroxytyrosol, oleic acid and coniferyl alcohol were selected as representative molecules of its phenolic, lipid and lignocellulosic fractions, respectively. Molecular dynamics simulations were used to investigate the interactions of representative olive pomace biocompounds with a bacterial membrane model, evaluating their membrane partitioning, molecular mobility and hydrogen-bonding patterns (Figure 1, right).

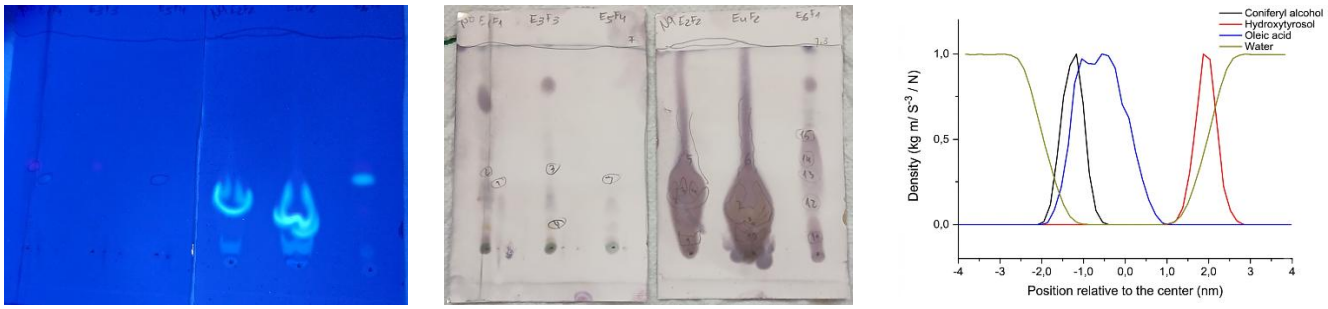


Figure 1 – Complementary experimental and computational characterization of olive pomace extracts. TLC fingerprinting under UV (left) and after vanillin/sulfuric acid derivatization (centre) highlights compositional differences among extracts obtained under different supercritical extraction conditions, while molecular dynamics simulations (right) reveal distinct membrane partitioning of representative biocompounds.

Overall, this work combines green extraction, chromatographic fingerprinting and molecular dynamics simulations into an integrated workflow for the sustainable valorisation of olive pomace. The proposed approach enables the selection of promising extraction conditions and provides molecular-level insight into the behaviour of representative biocompounds within bacterial membranes, supporting their future evaluation as natural agents against *Xylella fastidiosa*.

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Conventional and ultrasound-assisted extraction of *Castanea sativa* burs: A comparative study of phytochemical composition, bioactivity and cytotoxicity

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Agro-industrial by-products represent a valuable source of bioactive compounds, yet their valorisation depends on balancing extraction efficiency, biological activity and industrial feasibility. 1 This study compared conventional solid–liquid extraction and ultrasound-assisted extraction (UAE), using 80% ethanol and water as extraction solvents, to investigate the phytochemical composition and biological properties of Castanea sativa bur extracts and explore their potential for sustainable valorisation.

Four extracts (CSB1–CSB4) were characterized by LC-MS, revealing a phytochemical profile dominated by gallic acid together with flavonols such as rutin, quercetin glycosides, kaempferol derivatives and isorhamnetin derivatives. UAE generally yielded extracts richer in phenolic compounds, whereas conventional extraction remains a simpler and more readily scalable approach for industrial implementation.

Antioxidant capacity was assessed through DPPH, ABTS, FRAP, Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), with values ranging from 3.05 to 9.54 mmol TEAC/g lyophilized extract for DPPH, 1.61 to 5.75 mmol TEAC/g lyophilized extract for ABTS, 0.06 to 0.85 mmol TEAC/g lyophilized extract for FRAP, 127.65 to 838.97 mg GAE/g for TPC and 29.795 to 201.641 mg CTE/g for TFC.

All extracts exhibited antimicrobial activity against several foodborne pathogens, including Escherichia coli, Staphylococcus aureus and Listeria monocytogenes, with minimum inhibitory concentrations ranging from 0.781 mg/mL to >25 mg/mL.

Cytotoxicity was evaluated by the sulforhodamine B (SRB) assay after 72 h of exposure using HepG2, 3T3-L1, HFF-1, MRC-5, H9c2, Vero and SH-SY5Y cell lines. The extracts exhibited cell line-dependent cytotoxicity, with HepG2 cells being the most susceptible (GI_{50} = 18.02–156.82 µg/mL). Moderate cytotoxicity was also observed in MRC-5 and 3T3-L1 cells for selected extracts, whereas Vero, HFF-1 and SH-SY5Y cells were considerably less affected, with GI_{50} values exceeding 400 µg/mL in most cases.

The observed differences in cytotoxicity are likely associated with variations in the overall phytochemical composition and potential synergistic interactions among phenolic constituents, rather than with the abundance of individual compounds alone.2 Although the observed cytotoxicity may limit the direct use of these extracts as food ingredients or additives, it does not exclude their application in other technological approaches where consumer exposure is controlled. Accordingly, chestnut bur extracts represent promising green processing agents for cellulose and cellulose nanocrystal (CNC) isolation, enabling the development of reinforced biodegradable packaging materials for highly perishable foods, while further studies on compound migration and safety assessment would be required for food-contact applications.

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[P.5.25]

Grapeseed Oil: Influence of Raw Material and Extraction Method on Oil Yield and Vitamin E Content and Profile

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Grapeseed oil (GSO) is a source of vitamin E, including tocopherols and tocotrienols, lipophilic compounds that contribute to its nutritional quality and oxidative stability. This study aimed to characterize the variability in vitamin Ecomposition among commercial GSOs and to investigate how extraction method and raw material – grape seeds and pomace – affect oil yield and vitamin E. Three grapeseed lots were extracted with hot hexane (Soxhlet[1]), cold cyclohexane/isopropanol (Smedes method[2]), or by direct centrifugation, as a solvent-free laboratory-scale physical extraction method. Additionally, oils were obtained from six grape-pomace samples by direct centrifugation, and four commercial GSOs – three cold-pressed and one refined – were analyzed. Tocopherols and tocotrienols were quantified by normal-phase HPLC with fluorescence detection using validated laboratory method3.

Commercial cold-pressed GSOs showed a high variability on the vitamin E contents, ranging from 214 to 820 mg/kg, while the refined commercial oil contained 362 mg/kg oil of total vitamin E. Seed oil yields ranged from 7.0 to 11.9% for Soxhlet extraction, 6.6 to 9.3% for the Smedesmethod, and 1.5 to 4.6% for direct centrifugation. Across the three grape-seed lots, mean total vitamin E concentrations were 555, 670 and 686 mg/kg oil, within the range of the commercial samples. Both Smedes extraction and direct centrifugation produced oils with higher vitamin E concentrations than Soxhlet extraction (i.e., 23.8% and 27.5%, respectively), probably induced by the prolonged hot

extraction, although the magnitude of this difference was strongly lot dependent. Pomace-derived oils contained 313 – 590 mg/kg oil of total vitamin E. γ-Tocotrienol was the predominant homologue on all samples, accounting for 48.3 – 60.8% of total vitamin E, while γ-tocotrienol, α-tocopherol and α-tocotrienol together represented 86.4 – 92.7%. These results demonstrate that the raw-material and industrial-processing factors may exert a substantial influence on final oil composition.

Overall, direct centrifugation generated a vitamin E-rich oil fraction but provided lower oil recovery than solvent extraction. Its potential as a sustainable extraction strategy therefore depends on improving oil yield and demonstrating satisfactory vitamin E recovery on a seed or pomace basis.

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Antioxidant potential of chestnut burr extract: a circular approach to agro-industrial by-product valorization

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Chestnut harvesting and primary processing generate substantial amounts of chestnut burrs, the spiny lignocellulosic structures that naturally enclose the fruits. These agro-industrial by-products are frequently discarded or underused, despite their potential as sources of value-added bioactive constituents. The recovery of antioxidant fractions from this residual biomass may contribute to waste reduction, improved resource efficiency, and the development of sustainable products within a circular bioeconomy framework[1,2]. This study aimed to evaluate the antioxidant capacity of a chestnut burr extract as an initial step towards the sustainable valorization of this underused agro-industrial by-product.

Dried chestnut burr material was extracted at a solid-to-liquid ratio of 10 g per 200 mL using an 80% (v/v) ethanol-water solution.

Antioxidant capacity was evaluated using two complementary methods: the ferric reducing antioxidant power (FRAP) assay, which assesses electron-donating capacity, and the ABTS radical-scavenging assay, which evaluates the ability of the extract to neutralize the $ABTS^{\bullet+}$ radical cation. Trolox was used as the reference antioxidant, and the corresponding calibration curves showed high linearity, with coefficients of determination above 0.999.

The chestnut burr extract demonstrated antioxidant activity in both assays. The FRAP assay indicated a reducing capacity of 0.0154 mmol Trolox equivalents per gram of dry chestnut burr. This value was calculated considering the antioxidant-equivalent concentration of the extract, the total extraction volume of 200 mL, and the initial dry biomass mass of 10 g. In the ABTS assay, the extract reached a maximum radical-scavenging activity of 87.7% at a final concentration of 0.5 mg/ml. Although FRAP and ABTS assess distinct antioxidant mechanisms and their results cannot be directly compared, the combined findings demonstrate that the chestnut burr extract possesses both electron-donating and radical-scavenging capacity.

Overall, the results demonstrate the antioxidant potential of chestnut burr extract and support further investigation of this residual biomass within circular bioeconomy strategies. Its valorization may contribute to reducing the environmental burden associated with chestnut harvesting and primary processing while promoting the recovery of potentially valuable bioactive fractions. Further studies should include a detailed chemical characterization of the extract, the identification and quantification of the constituents contributing to its antioxidant activity, and the evaluation of its performance in relevant bio-based applications.

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WILD EDIBLE FRUITS OF CABINDA, MARKET AND TRADITIONAL KNOWLEDGE

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Wild and underutilized fruits are highly valued, widely consumed, and in high demand in informal local markets in Africa, then their trade constitutes a vital economic activity for indigenous rural inhabitants, playing a crucial role in supporting rural livelihoods[1]. Informal markets constitute privileged locations for ethnobotanical studies since they concentrate species from different ecosystems and bring vendors who possess traditional knowledge, allowing for the simultaneous documentation of the diversity of plant resources used, local knowledge systems, and their socioeconomic importance [2]. This is the first study that aims to document the diversity of wild and underutilized fruits sold in the informal markets of Cabinda through an ethnobotanical survey conducted between May 2023 and December 2024, using semi-structured interviews. Throughout the research, 310 vendors were interviewed, predominantly women (70%) aged between 15 and over 46 years old. The majority were Angolan nationals (74%), from 10 different provinces, mostly from Cabinda (80%). We identified twenty-three fruits belonging to 13 families, 23 genera, and 30 species. The Anacardiaceae family is the most representative followed by Malvaceae. The harvest season varies between species. In addition to food, some species are also used in traditional medicine for various categories of illnesses (gastrointestinal, respiratory, reproductive and urogenital health). The money earned from the sale of fruits is generally used to purchase basic needs. The Ethnobotanical and Economic Importance (IIEE) ranged from 0.0932 to 0.6627, revealing a wide heterogeneity in the relevance of the species marketed. The six species with the highest priority for conservation and market expansion were *Dacryodes edulis*, *Artocarpus camansi*, *Elaeis guineensis*, *Anisophyllea quangensis*, *Synsepalum dulcificum* and *Cola sp.*, all with values greater than 0.55. This study highlights the socio-economic and cultural role of wild fruits in Cabinda, also showed that the local markets can act as important hubs for transmission of traditional knowledge and sustainable development strategies[3].

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Purslane (*Portulaca oleracea L.*): Preliminary Instrumental and Sensory Characterization of Pancake Formulation for Early Childhood Nutrition

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Early childhood is a critical period for establishing healthy eating habits, highlighting the need for nutritious and sensory acceptable food products. Purslane (Portulaca oleracea L.), an underutilized edible plant naturally abundant in the Alentejo region (Portugal), is recognized for its high nutritional value and richness in bioactive compounds, making it a promising ingredient for the development of novel food products[1].

This study aimed to develop pancakes enriched with 10% purslane for potential application in early childhood nutrition and evaluate their sensory characteristics (Figure 1). Sensory acceptance was assessed by 58 untrained consumers (average age of 31 ± 16 years) using a 9-point hedonic scale, while the sensory profile was characterized by 35 untrained consumers (average age of 35 ± 16 years) using the Check-All-That-Apply (CATA) methodology. Adult consumers were used as sensory panellists because the study was designed as an initial product characterization, providing a preliminary assessment of acceptability prior to evaluation in the target population.

Both formulations achieved high consumer acceptance, with mean overall liking scores of 7.4 for the control and 6.6 for the purslane-enriched pancakes, with the difference between formulations being statistically significant ($p = 0.05$). Despite the lower mean score, the purslane-enriched formulation remained within the positive range of the hedonic scale. Texture was the best-rated attribute of the enriched formulation (7.5), demonstrating that the product maintained a soft and pleasant structure, an important characteristic for a product intended for young children. CATA analysis showed that purslane-enriched pancakes were mainly associated with green appearance (88%), herbaceous aroma (38%) and herbaceous flavour (44%), while retaining desirable texture attributes such as moist, soft and fluffy. Sweet taste from banana and oats contributed to balancing the vegetal characteristics, resulting in a sensory profile considered pleasant by consumers.

The incorporation of 10% purslane resulted in a clearly perceptible colour difference ($\Delta E = 4.65$), characterised by a statistically lower L, a, b* and chroma (C*) values, indicating a darker, less reddish and less saturated appearance compared with the control. In addition, the lower Browning Index (BI) suggested a reduced development of browning during cooking, indicating that the colour of the purslane-enriched pancakes was influenced not only by thermal reactions but also by the natural pigments present in purslane. These instrumental colour changes were consistent with the sensory evaluation, where most participants described the purslane-enriched pancakes as having a greenish appearance.

These findings highlight the technological feasibility of incorporating purslane into pancake formulations, providing a promising strategy for developing sensory acceptable food products incorporating a nutritionally valuable plant ingredient for potential application in early childhood nutrition. Further evaluation of the nutritional and functional properties of the final product, together with sensory studies involving the target age group, is needed to confirm its nutritional and functional potential and acceptability for young children.



Figure 1 - Appearance of the control and 10% purslane (*Portulaca oleracea L.*)-enriched pancake formulations.

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Phenolic Compounds as Chemical Markers of the Ageing Process of Wine Spirits

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The phenolic profile of wine spirit during ageing is significantly enriched with furanic derivatives, phenolic and volatile compounds, influencing its specific chemical and sensory characteristics.1 Wood extractives composition depends on several factors, such as botanical species, geographic origin, stave seasoning, toasting level, cellar conditions and the ageing technology[1]. While Limousin oak (Quercus robur L.) is traditionally used for WS ageing, chestnut (Castanea sativa Mill) wood has emerged as a sustainable alternative.1 Our research team has studied the low molecular weight (LMW) compounds transferred from wood extractives into WS using both traditional ageing technology (TAT; wooden barrels) and alternative ageing technology (AAT; wood staves combined with micro-oxygenation (MOX) in glass demijohns)[1]. AAT aims to simulate the traditional barrel process in a more sustainable way, faster, and cost-effective manner with lower environmental impact[1,2,3]. Studies characterizing the phenolic profile of aged wine spirits (WS) during storage in bottle obtained TAT and AAT remain scarce. Thus, this study (part of the Oxyrebrand project (https://projects.iniav.pt/oxyrebrand)) investigated the evolution of the phenolic profile (via HPLC-DAD-ESI-MS/MS) of WS aged with both botanical species (chestnut vs. oak) and technologies (TAT vs. AAT) over four years of bottle storage. The pilot-scale ageing trial used Portuguese chestnut and Limousin oak barrels (250 L) for TAT. For AAT, the MOX modality was applied to the WS during the ageing time, with pure oxygen supplied through a multiple diffuser micro-oxygenator (VISIO 6, Vivelys, France) with ceramic diffusers, at flow rate of 2 mL O_2/L /month during the first 60 days, followed by 0.6 L O_2/L/month of 61 until 365 days in 50 L glass demijohns with staves (chestnut vs oak staves)[1]. After 365 days of ageing process, all aged WS were bottled on the same day in 750mL amber glass bottles. The bottles were transported and stored in the cellar of INIAV (Polo de Dois Portos) at 19 ˚C and 80% relative humidity during 48 months[2]. The chromatographic profiles at 280 nm (Figure 1) of aged WS were similar between technologies for each botanical species after four years of bottle storage (oak wood (Figure 1-A) and chestnut wood (Figure 1-B)), confirming that AAT effectively mimics TAT regarding furanic aldehydes and phenolic profiles. Understanding this post-bottling variability is crucial, as it influences the choice of wood species and technology. The results highlighted the presence of gallotannins (mono-, di-, tri-, and tetragalloyl glucopyranose) and phenolic acids (gallic, protocatechuic, vanillic, syringic, ferulic and ellagic acids) in the WS aged with chestnut. The WS aged with Limousin oak showed the presence of ellagic acid glycosides and dimer (ellagic acid deoxyhexoside, ellagic acid pentoside and ellagic acid dimer) and phenolic acids (gallic, vanillic, syringic and ellagic acids). The phenolic aldehydes (vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde) were identified in WS aged from both wood botanical species. This study confirms the phenolic compounds as chemical markers of the ageing process of WS, as vanillin and syringaldehyde are commonly used as markers of the ageing process with wood, while gallic acid is a chemical marker of ageing with chestnut wood, and ellagic acid as a marker of ageing with oak wood[1,2,3].

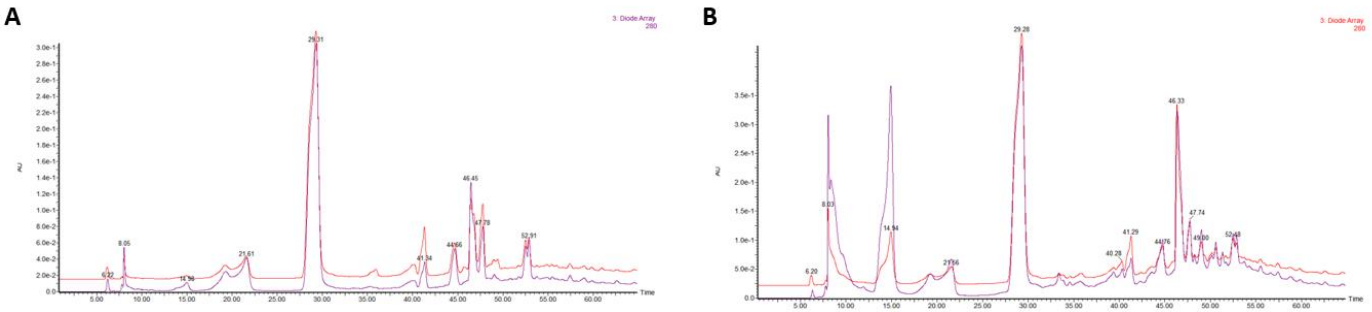


Figure 1 - Chromatographic profile at 280 nm of wine spirits aged using both ageing technologies (Traditional: purple colour; Alternative: red colour) after four years of storage in the bottle. (A) Limousin oak wood and (B) Portuguese chestnut wood.

In summary, the four-year bottle storage trial demonstrated that key compounds, such as hydrolysable tannins, phenolic acids and phenolic aldehydes, are maintained in a similar manner across both technologies. These findings are of crucial importance to the industry, as these compounds directly influence the organoleptic characteristics and overall consumers acceptability of aged wine spirits.

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Unlocking the potential of brewer's spent grain as a substrate for *Aspergillus oryzae* and edible mushrooms

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Aspergillus oryzae is a filamentous fungus traditionally used in Asian food fermentation and is known for its ability to produce hydrolytic enzymes, including endo-1,4-β-xylanases. These enzymes are involved in the degradation of xylan-rich lignocellulosic materials.

Brewer's spent grain (BSG) corresponds to the solid spent grain fraction retained after wort filtration during the lautering stage, it is the main solid by-product generated by the brewing industry in large quantities. Although it is commonly used as low-value animal feed, this material is mostly discarded or underused. Due to its high hemicellulose content, BSG could be a suitable substrate for fungal growth and the production of value-added compounds.

The aim of this study was to evaluate whether BSG could be used as an alternative substrate for the growth of *A. oryzae*, as a first step towards the production of xylooligosaccharides.

Six BSG samples, designated BSG1–BSG6, originate from distinct grist compositions: just malted barley, malted barley and wheat, and malted barley and rye. Three additional BSG samples include adjuncts (non malt sources of fermentable sugars) incorporated during wort production (maize, oat flakes and wheat flakes). Whole rice flour, the traditional substrate used for koji production, was included as the reference substrate.

Commercial Koji-kin, consisting of *A. oryzae* spores mixed with rice flour, was used as the initial source of spores. Spores were recovered using saline solution containing Tween 80 and inoculated onto potato dextrose agar (PDA). After incubation at 26.5 °C for one week, spores were collected from the plates using the same solution, and the suspension was adjusted to 6×10^6 spores/mL. BSG and rice flour suspensions were prepared at 1% (w/v) in sterile distilled water and inoculated with the spore suspension. Cultures were incubated at 30 °C and 140 rpm, and fungal growth was visually assessed after 24, 48 and 72 h.

Preliminary results showed that *A. oryzae* was able to grow on most BSG samples. However, differences in mycelial growth and sporulation were observed among the different formulations. At the same time, further studies will evaluate xylanase production and the conversion of BSG hemicellulose into xylooligosaccharides during *A. oryzae* growth.

In a parallel experiment, one of the BSG samples was mixed with pinewood pellets for the cultivation of several species of *Pleurotus* (*ostreatus*, *citrinopileatus* and *djamor*), *Lentinula edodes* (shiitake) and *Hericium erinaceus* (Lion's mane). All *Pleurotus* fully colonized the substrate in less than one week; Shiitakes and Lion´s mane, having less vigorous mycelium, show a slower development. This trend has also been observed in other substrates, as *Pleurotus* species are generally more vigorous and therefore exhibit faster growth.

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The new miracle of roses: roses in gastronomy and their impact on health

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Roses are historically linked to the city of Coimbra through the miracle of the transformation of bread into roses in the lap of Queen Saint Isabel of Portugal, who lived in the city.
Today, the miracle occurs in the opposite direction, with roses being transformed into edible products used in gastronomy. Although the use of roses as food is not a traditional practice in Portugal, this approach creates opportunities for the valorization of natural resources, territory, economy and cultural heritage.
This work presents a review and reflection on roses from biological, cultural, spiritual, medicinal, religious, historical and literary perspectives, with particular emphasis on their use as edible flowers. The study also addresses the natural colors of roses as an alternative to artificial food colorants, their chemical composition, pharmacological properties and nutritional and functional importance. The methodology was based on the analysis of scientific articles published in specialized journals, complemented by experience developed in the field.
Particular attention is given to products developed from roses and their potential applications in gastronomy and food innovation. Edible roses provide an attractive element in food presentation and constitute a source of phytochemicals with potential benefits for health. Their incorporation into foods may therefore contribute to the development of functional foods and open new market opportunities in Portugal. Coimbra provides an appropriate context for further research and the development of new studies on the food, functional and cultural potential of roses.

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Effect of Mechanical Extraction Conditions on the Chemical Composition and Oxidative Stability of *Tenebrio molitor* Oil

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Insect farming is increasingly regarded as a more resource-efficient alternative to conventional animal protein production, requiring markedly less water and agricultural land than livestock farming, which remains a major driver of natural resource depletion.1 Among edible insects, Tenebrio molitor(mealworm) larvae combine a favourable nutritional profile with efficient feed conversion efficiency[2]. Their processing for protein concentrate production requires a preliminary defatting step, from which an oil fraction is recovered as a co-product; despite its high content in unsaturated fatty acids, this fraction remains comparatively under-valorised[3]. This work aimed to establish how the conditions used for the mechanical extraction of T. molitor oil affect its chemical composition and oxidative behaviour, thereby supporting the selection of a suitable, scalable processing route.

Oil samples were obtained by solvent-free hydraulic pressing of T. molitor flour, combining three temperatures (40, 70 and 95 °C) with four pressing durations (5, 15, 30 and 45 min). Fatty acid composition was determined by Gas Chromatography-Flame Ionization Detection (GC-FID); primary and secondary oxidation were assessed through peroxide value (PV) and thiobarbituric acid reactive substances (TBARS), respectively, complemented by differential scanning calorimetry (DSC, 25-300 °C, 55 min).

Oxidative and colour stability of the oils obtained under each extraction condition were further monitored during a 15-day accelerated oxidation assay (Schaal Oven Test, 60 °C, four sampling points), through PV, TBARS and CIELAB colour parameters (ΔE*ab). Oleic acid predominated in all samples, followed by linoleic and palmitic acids; the fatty acid profile, together with a consistently low omega-3 content (resulting in relatively high omega-6/omega-3 ratios of 44-48), was unaffected by the pressing temperature or duration tested. Already before accelerated oxidation, oils pressed at 40 °C displayed a higher PV (~7.1 vs 3.2-4.0 meq O_2/kg oil) and a more negative a* value (greener hue) than oils pressed at 70 or 95 °C, whereas baseline TBARS did not differ between conditions. This lower initial oxidative quality at 40 °C was corroborated by an earlier DSC oxidation onset, and confirmed throughout accelerated oxidation, during which the 40 °C oils consistently exhibited the highest PV and TBARS values and the most pronounced colour shift towards green; within each temperature, however, differences among pressing durations within the same temperature remained limited throughout the 15-day storage period. These results indicate that pressing at 70 or 95 °C yields oils with better oxidative and chromatic stability than pressing at 40 °C, without compromising fatty acid composition, and should therefore be favoured over the latter in subsequent processing. Ongoing work is directed at purifying the selected oil to a food-grade quality while preserving its bioactive and functional compounds. Altogether, these findings support solvent-free mechanical pressing as a scalable strategy to convert an under-valorised insect processing fraction into a chemically stable, functional lipid ingredient, promoting a more circular food system while providing a more sustainable extraction approach.

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XVIII Encontro de Química dos Alimentos

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ALIMENTOS TRADICIONAIS E VALORIZAÇÃO DO PATRIMÓNIO ALIMENTAR



Nasceu em São Tomé, em 1957, e desenvolveu um percurso profissional diversificado nas áreas da cultura, arte, gastronomia, comunicação e desenvolvimento territorial. Artista plástico e antigo jornalista, fundou o Centro Internacional de Arte e Cultura e o Espaço Teia D'Arte, em São Tomé, e é fundador e coordenador da Bienal de Arte e Cultura de São Tomé e Príncipe. Desenvolve o projeto integrado de desenvolvimento da Roça São João, que articula agricultura, ambiente, património, cultura, educação e turismo, e criou projetos como a OLGA – Oficina de Laboratório de Gastronomia de Angolares e o FOLGA – Festival da OLGA. Foi autor e apresentador de vários programas de televisão da RTP dedicados à gastronomia, cultura e valorização dos produtos locais. Em 2008 foi nomeado Embaixador da Cultura de São Tomé e Príncipe e, em 2019, recebeu o Diploma de Mérito pelos serviços prestados à Nação. Atualmente, continua a desenvolver projetos culturais, artísticos, gastronómicos e de valorização do património são-tomense.

Cozinha social ancestral

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Cozinha Social Ancestral de Água-Izé

Abertura — o que é o projeto

A Cozinha Social Ancestral de Água-Izé é uma das componentes estruturantes do programa Água-Izé Reinventada, promovido pela Roça Mundo em São Tomé e Príncipe. Nasce da visão traçada no Seminário Internacional reInventar as Roças: Água Izé, em 2023.

A gastronomia é usada como instrumento de preservação patrimonial, capacitação comunitária e criação de rendimento. Não é um projeto de restauração convencional, é pensado como infraestrutura de hospitalidade cultural, que junta formação, experimentação gastronómica, mediação cultural e valorização da produção local.

Onde e como

Instala-se na antiga Fábrica de Óleo de Palma, um edifício patrimonial a recuperar. Vai ter cozinha profissional, cozinha tradicional exterior junto às hortas pedagógicas, espaços de oficinas culinárias e sala de refeições. A cozinha Social e Ancestral, revisita toda a gastronomia dos povos que estão na base da formação histórica e cultural do Santomense.

A ligação ao resto do território

Articula-se diretamente com o Núcleo Museológico do Cacau e com a Unidade de Residências Artísticas e de Investigação Científica. Isto prolonga a estadia dos visitantes e reforça todo o programa Água-Izé Reinventada.

O modelo de sustentabilidade

Assenta na diversificação de receitas para não depender de uma única fonte: experiências gastronómicas participativas, oficinas, formação, apoio alimentar às residências, catering, eventos e venda de produtos locais. Aposta em cadeias curtas com produtores locais.

E vai alimentar um jardim de infância e uma escola primária em Água Izé, num total de 330 crianças. Uma parte da receita servirá para apoio a manutenção das duas escolas.

Os números

Investimento total de 120 000 €, a mobilizar por capital próprio, financiamento e apoios externos de cooperação. Horizonte de implementação de três anos.

Fecho — a mensagem chave

É um projeto que transforma património e saber alimentar em oportunidade real para a comunidade de Água-Izé, ligando tradição, formação e economia local.

Development of a 3D Printed Octopus Based Snack Inspired by the Traditional "Pulpo a la Gallega": Nutritional Characterization and Sensory Evaluation

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Three dimensional (3D) food printing has emerged as an innovative technology for developing customised foods with tailored nutritional composition and appealing structures[1,2]. Combining this technology with traditional gastronomy offers an attractive strategy to create novel products while preserving cultural identity and promoting the valorisation of marine resources. In this study, a 3D printed snack inspired by the traditional Spanish dish *Pulpo a la Gallega* was developed using octopus (*Octopus vulgaris*) as the main ingredient. The objective was to evaluate the nutritional composition, mineral profile and sensory evaluation of the final product. Two print able formulations were prepared: a potato-based matrix and an octopus-based matrix. The final snack (**Figure 1**) was produced by sequential layer deposition using extrusion based 3D printing with a Foodini® food printer (Natural Machines), with the potato formulation forming the bottom layer and the octopus formulation the top layer. Proximate composition, including moisture, protein, fat, ash, dietary fibre, carbohydrates and energy value, was determined using standard analytical methods. Mineral composition was also evaluated. Sensory evaluation was carried out by an untrained consumer panel of 40 participants (23–76 years old; 25 women and 15 men), assessing appearance, odour, flavour, texture, overall liking and purchase intention.



Figure 1 - 3D-printed octopus-based snack inspired by *Pulpo a la Gallega*.

The incorporation of the octopus based formulation significantly improved the nutritional quality of the snack. While the potato base contained only 3.1 g protein/100 g, the octopus base provided 21.5 g/100 g, resulting in a final product with 12.9 g protein/100 g. Proteins accounted for 42% of the total energy value, allowing the product to comply with the requirements for the nutritional claim "high protein" established by Regulation (EC) No. 1924/2006[3]. The snack also presented lower carbohydrate and fat content and higher ash values. Mineral analysis demonstrated that the incorporation of octopus contributed to the mineral composition of the snack, particularly by increasing the levels of potassium, phosphorus, sulphur and zinc. These findings highlight the potential of marine ingredients to improve the nutritional quality of 3D printed foods while maintaining their technological suitability for extrusion based printing. Sensory evaluation revealed favourable consumer acceptance, with balanced scores for appearance, odour, flavour, texture and overall liking. Purchase intention was also encouraging, with more than 67% of participants indicating that they would probably or certainly buy the product. These findings demonstrate that the incorporation of octopus enhanced the nutritional value of the snack while preserving desirable sensory characteristics.

Overall, this work demonstrates that combining extrusion based 3D food printing with inspiration from traditional Mediterranean gastronomy enables the development of innovative seafood based snacks with high nutritional value and good consumer acceptance. This approach contributes to the diversification and valorisation of marine resources and represents a promising strategy for the development of next-generation functional foods that combine technological innovation, nutritional quality and gastronomic heritage.

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Sustainable bioprocessing strategies to enhance the functional potential of underexplored Mediterranean legumes

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Lentils (Lens culinaris) and lupin (Lupinus albus) are traditional Mediterranean legumes that have long been part of regional diets and represent valuable sources of proteins, dietary fibre, and phenolic compounds. Despite their nutritional benefits, the full functional potential of these legumes remains underexploited due to the limited bioaccessibility of bioactive compounds and the presence of anti-nutritional factors (ANFs), such as phytic acid (PA) and quinolizidine alkaloids [1, 2]. Sustainable bioprocessing technologies have therefore emerged as valuable approaches to improve the nutritional quality and biological functionality of legumes. Among these, germination is a simple, cost-effective, and environmentally friendly process capable of activating endogenous enzymes, promoting the synthesis and release of bioactive compounds, and simultaneously reducing ANFs. This study evaluated the effects of controlled germination (temperature and time) on the functional properties of lentils and lupin by assessing total phenolic content (TPC), antioxidant capacity (DPPH, ABTS, FRAP, and ORAC), antidiabetic, antihypertensive and anti-hypercholesterolemic potential, and ANFs.

Germination (20 ºC, 95% humidity) significantly modulated the bioactive profile of both legumes in a species-dependent manner. Lentils exhibited the most pronounced response after 24 h of germination, with TPC increasing from approximately 2.7 ± 0.2 to 3.6 ± 0.1 mg GAE/g DW ($p < 0.01$). These values were complemented by enhanced antioxidant activity, particularly in the ORAC assay (69.3 ± 4.3 to 89.1 ± 8.4 µmol TE/g DW) and FRAP (28.8 ± 1.0 to 37.5 ± 1.8 µmol TE/g DW), suggesting that germination promoted the increase and/or improved accessibility of antioxidant compounds. Lupin showed the greatest improvements after 48 h of germination, with TPC increasing from 0.87 ± 0.03 to 1.35 ± 0.13 mg GAE/g DW ($p < 0.05$), together with higher antioxidant capacity, especially ORAC values, which increased from 20.6 ± 1.98 to 30.5 ± 2.10 µmol TE/g DW. The different optimal germination times highlight species-specific metabolic responses during seed sprouting.

Beyond antioxidant activity, germination also improved the metabolic health-related properties of both legumes. Lentils exhibited a marked enhancement of α-glucosidase inhibitory activity, with inhibition increasing from approximately 50% in non-germinated seeds to over 90% after germination at 10 mg/mL. This was reflected in a substantial reduction of the IC_{50}, from 11.81 mg/mL in control seeds to 1.47, 1.74 and 3.24 mg/mL after 16, 24 and 48 h of germination, respectively. Lupin also showed improved α-glucosidase inhibition, increasing from approximately 5% in control seeds to 18% after 48 h of germination. Likewise, Angiotensin-Converting Enzyme (ACE) inhibitory activity was significantly enhanced in both species. In lentils, inhibition increased from approximately 10% in control seeds to 36–40% after 16–24 h of germination, while lupin increased from around 11% to 25% after 48 h. These findings indicate that germination enhances not only antioxidant properties but also the antidiabetic and antihypertensive potential of Mediterranean legumes. Preliminary analyses further suggest an increase in cholesterol-lowering potential following germination. Regarding ANFs, PA content decreased markedly in lentils, from 30.1 ± 0.81 to 22.4 ± 0.70 mg/g DW after 24 h of germination, while more moderate reductions were observed in lupin. Preliminary analyses also indicate a reduction in lupin alkaloids, particularly lupanine, further improving its nutritional quality.

Overall, these findings demonstrate that germination represents an effective strategy to enhance the nutritional quality and functional potential of Mediterranean legumes by increasing phenolic compounds and health-related bioactivities while reducing ANFs. Therefore, germination may contribute to the development of innovative plant-based ingredients with added nutritional and functional value. Future work will combine detailed phenolic profiling with the evaluation of solid-state fermentation, allowing a direct comparison between both bioprocessing strategies to identify the most effective approach for maximizing bioactive compound availability and health-promoting properties.

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Effect of Pulsed Electric Fields on Microbial Inactivation and Stability of Strawberry Tree Juice during Refrigerated Storage

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Escherichia coli is a Gram-negative microorganism widely associated with foodborne contamination, representing a significant safety concern in fruit-based products due to its ability to survive under acidic conditions and potentially recover during storage[1]. Despite its nutritional potential, strawberry tree (*Arbutus unedo* L.), a Mediterranean specie, remains underexploited due to its high perishability and handling limitations[2]. Pulsed electric fields (PEF) (HPP) has been applied as a nonthermal pasteurization technology, enabling effective microbial inactivation while preserving product quality. However, its efficiency depends on process conditions and matrix characteristics, requiring further investigation to ensure microbial stability during refrigerated storage.

This study aimed to evaluate the effectiveness of HPP on microbial inactivation and shelf-life stability of strawberry tree (*Arbutus unedo* L.) juice (pH 3.02 ± 0.01 and °Brix 14.50 ± 0.00) during refrigerated storage (5 ºC) for 30 days and compare it with thermal pasteurization. Experimental analyses were conducted on both inoculated samples with *Escherichia coli* (used as a target pathogenic surrogated microorganism to optimize the thermal and nonthermal pasteurization conditions) and on the juices' endogenous microorganisms (namely total aerobic mesophiles, psychrophiles and yeasts and moulds). PEF treatments (12 – 15 and, 17.5 KV/cm, one and two cycles at temperature between 35 – 65 °C), were applied and compared with conventional thermal pasteurization (70 °C for 5-30 min). Additionally, challenge tests using pre-deteriorated (purposedly spoiled juice kept at room temperature for 16 days to induce microbial growth) were performed simulate a scenario of a juice heavily contaminated to assess the maximum microbial inactivation capacity of each treatment.

PEF treatments achieved more than 6-log units' inactivation of *E. coli* below detection limits under two cycles of 17.5 KV/cm combined with pre-treatment at 60°C, similarly to thermal pasteurization (at 70 °C for 5, 10, 20 and 30 min).

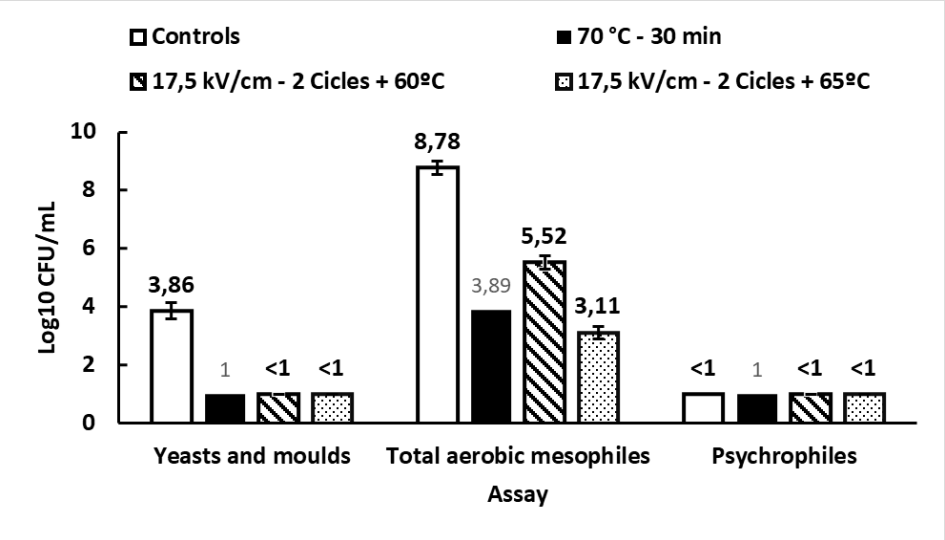


Figure 1 - Microbial loads of yeasts and moulds, total aerobic mesophiles, and psychrophiles expressed as the decimal logarithm of colony forming units per mL of strawberry tree juice (Log10 CFU/mL) in spoiled juice (stored for 16 days at room temperature prior to processing), used to assess the impact of PEF combined with moderate temperature and thermal pasteurization (70 ºC, 30 min) on the inactivation of indigenous microorganisms.

When it comes to the challenge tests with spoiled juice, the inactivation levels of total aerobic mesophiles and yeasts and moulds were directly proportional to the pre-treatment temperature applied, i.e., higher temperature (≥60 °C) resulted in higher microbial inactivation (with more than 5 log units' reduction), but still with a low residual fraction, which remained

within acceptable hygiene thresholds, while thermal pasteurization (70 °C for 30 min) was unable to reach the 5-log units reduction (Figure 1). This contrasts the feasibility of synergy between PEF and temperature to achieve higher microbial loads reductions in shorter processing times when compared to thermal pasteurization.

In natural juice (containing only its indigenous fraction of microorganisms), both treatments (thermal pasteurization and two cycles PEF 17.5 KV/cm + 65°C) initially reduced microbial loads to below detection limits. Moreover, during 30 days of refrigerated storage, both treatments maintained microbial stability, with total aerobic mesophiles, psychrophiles and yeasts and moulds remaining below detection limits.

Overall, PEF combined with moderate thermal pre-treatment proved to be an effective treatment alternative to conventional thermal pasteurization, ensuring both microbial safety and extended shelf-life in strawberry tree juice, in shorter processing times. These findings support the potential application of PEF in the preservation of high-value fruit juices.

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Reinvention of portuguese seafood rice: a plant-based approach based on the synergy between polysaccharide hydrocolloids and seaweeds

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The increasing demand for plant-based foods is driven by multiple and interrelated factors, including health considerations, environmental sustainability, ethical concerns, and the need to accommodate vegan and allergen-sensitive consumers.[1] In parallel, the seafood sector is under growing pressure due to overfishing, habitat degradation, biodiversity loss, and the broader impacts of climate change and inefficient resource management. Within this context, plant-based seafood analogues have emerged as a promising strategy to reduce pressure on aquatic ecosystems while maintaining familiar sensory and cultural food experiences. However, the successful reformulation of seafood-based dishes remains challenging, particularly due to the need to reproduce the characteristic fibrous-gel structure, high water content, and umami-rich flavour profile of marine muscle proteins, which are largely associated with non-volatile compounds such as free glutamic acid salts and 5'-nucleotides.[2]

This study aimed to develop a plant-based reinterpretation of the traditional Portuguese “Arroz de Marisco” (seafood rice), a dish with strong cultural identity and historical relevance, reflecting both high national rice consumption and among the highest per capita fish consumption rates.[3] In addition, sensory evaluation was conducted using a focus group with six untrained panellists.

Plant-based shrimp and fish analogues were formulated and optimized using of polysaccharide hydrocolloid and seaweed ingredients to recreate seafood-like textures, flavours, and visual attributes. Specifically, combinations of xanthan gum, gellan gum, agar-agar, and iota-carrageenan were employed to mimic the elastic and gel-like structure of shrimp and fish analogues. These hydrocolloids were selected due to their complementary rheological properties, including gel formation, elasticity modulation, and water-binding capacity, which are essential for simulating seafood texture. In parallel, seaweed-based systems including kombu (Saccharina japonica), sea lettuce (Ulva spp.), and nori (Porphyra spp.) were incorporated as natural sources of umami compounds and marine aroma precursors. The final formulation successfully integrated texture, aroma, and visual appearance achieving mean sensory scores above 8, through the combined use of seaweed infusions and polysaccharide hydrocolloids. This resulted in a plant-based seafood rice with high sensory acceptance and organoleptic characteristics closely resembling those of the traditional dish. In addition, the reformulation contributes to a more inclusive dietary profile by reducing exposure to common fish and shellfish allergens. Beyond technological performance, this work highlights the potential of combining structured hydrocolloid systems with marine-derived plant ingredients as a viable strategy for recreating complex seafood sensory experiences. The approach supports the development of sustainable gastronomic innovations that preserve culinary heritage while addressing contemporary challenges related to environmental impact. Overall, the study highlights the potential of combining hydrocolloid structuring systems with seaweed ingredients to recreate seafood-like sensory profiles, supporting sustainable and inclusive innovation in traditional relevant dishes.

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Nutritional characterization of Talinum paniculatum (Jacq.) Gaertn. seed flour and its application as a partial wheat flour substitute in muffins

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The growing interest in alternative ingredients in the food industry has driven research into plant-based ingredients with high nutritional value. Talinum paniculatum (Jacq.) Gaertn., a plant widely distributed in Brazil and classified as an Unconventional Food Plant (UFP), has been reported as a promising source of nutrients and has demonstrated antioxidant and antimicrobial activities[1]. However, its seeds remain poorly explored as food ingredients. Thus, this study aimed to evaluate the nutritional composition and technological properties of T. paniculatum seed flour and its effect as a partial wheat flour substitute on the physicochemical and textural properties of muffins. Seeds were collected in Medianeira, Paraná, Brazil, cleaned to remove impurities, transported under vacuum to Bragança, Portugal, and then milled to obtain flour. Moisture, ash, lipid, protein, and total dietary fiber analyses were conducted in triplicate following AOAC official methods, whereas carbohydrate content was calculated by difference. Additionally, water-holding capacity (WHC) and oil-binding capacity (OBC) were determined in triplicate[2]. Muffins were prepared according to a conventional recipe, using wheat flour as the control and replacing it with T. paniculatum seed flour at 5%, 10%, and 15%. The batter was prepared using a Thermomix® TM6, and muffins were baked in a convection oven at 140 °C for 15 min. After baking, colour was evaluated by instrumental colorimetry (CIELAB: L*, a*, b*) using a Konica Minolta CR-400 colorimeter; water activity was measured by direct dew point reading (Novasina LabStart-aw); moisture was determined using an ADAM® PMB163 moisture meter; and texture profile was assessed instrumentally with a TA.XTPlus texture analyser (36 mm probe, 5 kg load cell).

The nutritional characterization of T. paniculatum seeds revealed a moisture content of 12.42 ± 0.20 g/100 g. On a dry weight basis, total dietary fiber was the major macronutrient (54.17 ± 1.11 g/100 g), followed by proteins (23.92 ± 0.30 g/100 g), lipids (12.24 ± 0.87 g/100 g), carbohydrates (6.17 ± 1.43 g/100 g), and ash (3.00 ± 0.16 g/100 g), corresponding to an energy value of 342.96 ± 2.54 kcal/100 g. These findings highlight seeds as a notably fiber- and protein-rich matrix with potential relevance for food fortification. T. paniculatum seed flour presented a WHC of 185.32 ± 2.87% and an OBC of 123.96 ± 3.59%, indicating relevant technological potential for application in bakery products. The incorporation of increasing levels (5%, 10%, and 15%) of T. paniculatum produced consistent changes in the physicochemical and textural properties of muffins compared with the wheat-flour control. Moisture and water activity increased with T. paniculatum substitution level, reaching 47.1 ± 0.51% and 0.91 ± 0.02 at 15%, respectively, compared with 23.1 ± 1.03% and 0.86 ± 0.04 in the control. Colour analysis revealed progressive darkening and a shift towards redder and less yellow tones, with L* decreasing from 64.2 ± 1.77 (control) to 38.5 ± 1.26 (15%).

Texture parameters such as firmness, hardness, gumminess and chewiness generally decreased in muffins containing T. paniculatum flour, particularly at 10% substitution, whereas springiness, cohesiveness and resilience remained relatively stable across formulations. The 10% substitution led to the most pronounced reduction in mechanical resistance, while 15% produced the highest moisture and water activity values and the most marked colour changes. The stability of springiness, cohesiveness and resilience suggests that crumb structural integrity is preserved regardless of substitution level. Overall, T. paniculatum seed flour showed high fiber and protein contents, relevant water- and oil-interaction properties, and potential for partial wheat flour substitution in muffin formulations. Further studies, including bioactivity and sensory evaluation, are underway to assess consumer acceptance and determine the most suitable incorporation level.

Acknowledgments: This work was supported by national funds from Fundação para a Ciência e Tecnologia (FCT/MCTES-PIDDAC) through the base funding UIDB/00690/2025 and UIDP/00690/2025; and from SusTEC, LA/P/0007/2020. L. B. Barros acknowledges FCT through the institutional scientific employment program-contract (CEECINST/00107/2021/CP2793/CT0002).

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Green Alchemy: Transforming Avocado Waste into Efficient Water Treatment

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The increasing demand for sustainable wastewater treatment technologies has driven the search for bio-based alternatives to conventional inorganic coagulants. Among these, tannins have emerged as promising natural compounds due to their polyphenolic structure, biodegradability, and ability to destabilize colloidal particles [1]. This work investigates the potential of tannin-based coagulants derived from avocado seed, an abundant agro-industrial residue, for application in food industry wastewater treatment [2]. Tannins were extracted through aqueous solid–liquid extraction under optimized conditions to maximize the recovery of condensed tannins. The extracts were characterized in terms of total phenolic content and tannin concentration and subsequently modified via Mannichreaction to produce cationised tannins with enhanced charge density, aiming to improve coagulation performance [3]. The coagulation efficiency of both natural and modified tannin extracts was evaluated through jar test experiments using real wastewater. The untreated tannin extracts showed limited performance as primary coagulants, leading in some cases to increased turbidity, which may be attributed to their intrinsic coloration and insufficient charge neutralization capacity under the tested conditions. In contrast, cationised tannins demonstrated improved performance when used in combination with coagulant aids. The addition of $CaCl_2$ significantly enhanced turbidity removal, reaching up to 70%, while the combined use of $CaCl_2$ and flocculants achieved removal efficiencies of up to 72%. These results highlight the importance of system conditions, such as ionic strength and the presence of bridging agents, in promoting the coagulation activity of tannin-based materials. Despite the moderate performance, tannin-based coagulants present important advantages in terms of sustainability, including renewability, low toxicity, and potential integration into circular economy strategies through the valorisation of agro-industrial waste. However, their effectiveness is strongly dependent on chemical modification and process conditions, suggesting that further optimization of functionalisation (e.g., degree of cationisation) is required to enhance their applicability as primary coagulants.

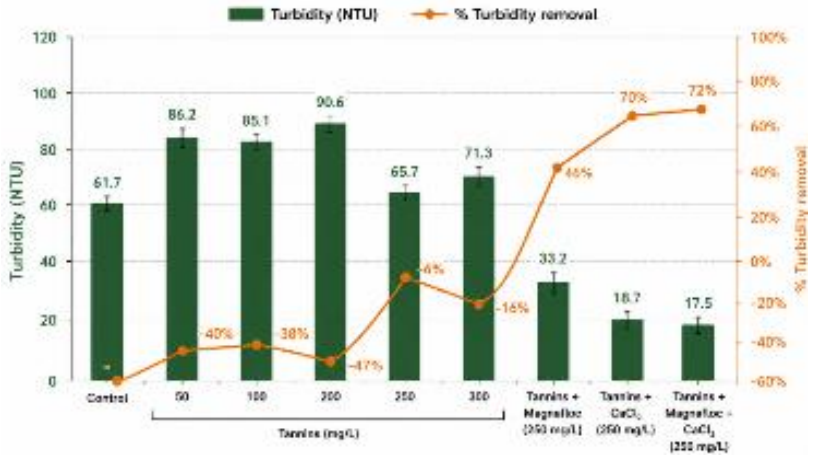


Figure 1 - Effect of tannin concentration and coagulant aids (Magnafloc and $CaCl_2$) on turbidity removal efficiency during coagulation–flocculation of food industry wastewater.

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Processing Effects on Carbohydrate Digestibility and Nutritional Properties of Rice Flours

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Hydrolysed flours are increasingly incorporated into infant and functional foods to enhance digestibility and sweetness, yet its nutritional impact may depend on gastrointestinal maturity. This study assessed carbohydrate release profiles of hydrolysed versus non-hydrolysed rice-based flours (HF, NHF) and rice-based baby instant meals (HM, NHM), following both adult and infant in vitro digestion protocols to evaluate whether i) gastrointestinal maturity, ii) flour pre-hydrolysis, and iii) product format influence carbohydrate breakdown.

Samples were prepared according to industrial specifications and subjected to triplicate digestion under standard adult INFOGEST 2.0 conditions and an infant-adapted protocol representing 4-month-old physiology with age-specific enzyme concentrations, incubation time, and pH. Soluble carbohydrate profiles and time-resolved glucose release were determined by HPLC-RI.

Digestion efficiency was consistently higher under adult conditions across all matrices, evidenced by greater monosaccharide release and lower residual starch. Infant-model digestions, by contrast, showed higher maltose proportions (~50%), reduced glucose (12.4-19.8%), and greater persistence of starch and longer-chain oligosaccharides, reflecting comparatively limited enzymatic activity.

Industrial hydrolysis consistently shifted the carbohydrate profile towards less polymerised saccharides, compared with non-hydrolysed samples. In the adult model, glucose release increased significantly with preparation (meal versus flour) in non-hydrolysed samples, whereas this increase was only marginal in hydrolysed samples, indicating that most of the conversion had already occurred during industrial processing. In the infant model, hydrolysis promoted a clear increased glucose release, but preparation tended to reduce this effect, possibly reflecting structural limitations and shorter digestion time. Overall, digestive physiology was the dominant determinant of the final carbohydrate profile ($F = 277.2$, $p < 0.001$), but a strong hydrolysis × product-format interaction was also observed ($F = 191.3$, $p < 0.001$).

Product format had a greater influence under infant digestive conditions, suggesting that the physical structure (i.e., viscosity) modulates carbohydrate digestion when enzymatic capacity is limited. In flours, unlike instant meals, industrial hydrolysis led to a relative increase in intermediate oligosaccharides, suggesting their accumulation due to insufficient downstream enzymatic breakdown.

Overall, these findings demonstrate that carbohydrate digestibility is determined by the combined effects of industrial hydrolysis, digestive maturity, and product format, emphasising the need to tailor processing strategies to the physiological capacity of the target population, with direct implications for infant food formulation.

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Nutritional Composition of Commercially Important Marine Fish Species

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The Mediterranean diet is globally recognised as one of the healthiest dietary patterns, being characterised by a high consumption of fish, olive oil, vegetables, legumes and whole grains. Within this dietary framework, marine fish species, particularly small pelagics, play a key role as a primary source of high-quality protein and omega-3 long-chain polyunsaturated fatty acids (LC-PUFA), as well as essential micronutrients difficult to find in many other foods.1,2 Despite their nutritional, economic and cultural importance, there is a lack of comparative data on the nutritional profiles of this important species on the Iberian Peninsula.

This study aimed to compare the proximate composition of fillets from commercially relevant marine fish species. Due to the body size of some species, the number of fish required to obtain sufficient material for each analytical unit varied, resulting in the following sampling structure: European anchovy (Engraulis encrasicolus) - 82 fish yielding 2 composite samples; European sardine (Sardina pilchardus) - 27 fish yielding 3 samples; Atlantic chub mackerel (Scomber colias) - 6 fish yielding 2 samples; Atlantic mackerel (Scomber scombrus) - 6 fish yielding 2 samples; and round sardinella (Sardinella aurita) - 4 fish yielding 4 samples. These species are predominantly caught by purse-seine fisheries operating in Portugal, Spain and the wider Mediterranean region, where they are particularly important and widely consumed. The samples (n = 132) were prepared and analysed in duplicate at the Animal Nutrition and Feeding Laboratory (LNAA) of the School of Agriculture, Polytechnic University of Castelo Branco (ESA-IUPCB). Individual specimens were measured, weighed, filleted to obtain the edible muscle fraction, and homogenised. Proximate composition analyses were conducted to determine moisture (NP 2282:2009), ash (NP 2032:2009), crude protein using the Kjeldahl method (NP 4488:2009), and crude fat using the Soxtec method with petroleum ether (NP 1974:2009). Statistical analyses were performed in SPSS. Interspecific differences were tested using one-way ANOVA followed by the Tukey–Kramer multiple comparison test ($p < 0.05$). All species exhibited a favourable nutritional profile consistent with their classification as functional foods within the Mediterranean diet (Figure 1).

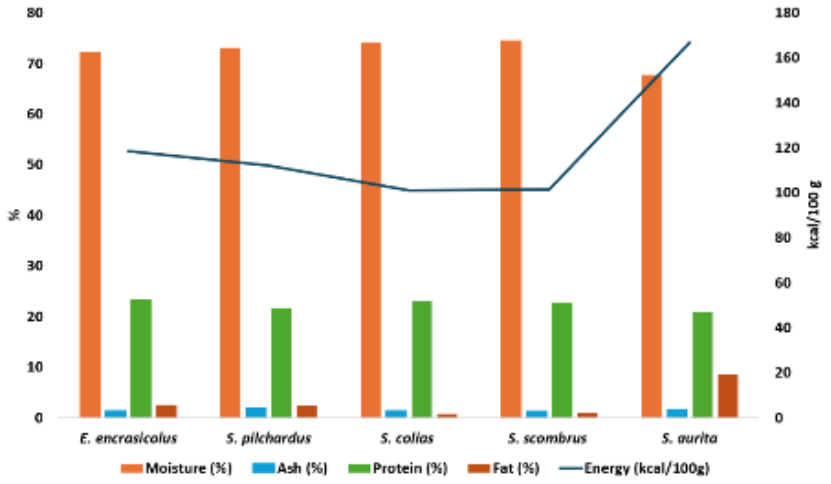


Figure 1 – Nutritional composition of *Engraulis encrasicolus, Sardina pilchardus, Scomber colias, Scomber scombrus and Sardinella aurita.*

Protein content was consistently high across species, ranging from 20.80% in *S. aurita* to 23.42% in *E. encrasicolus*. Moisture ranged from 67.75% ($p < 0.05$) in *S. aurita* to 74.57% in *S. scombrus*, while ash content remained low and relatively stable, varying between 1.35% in *S. scombrus* and 1.96% ($p < 0.05$) in *S. pilchardus*. Crude fat showed marked variability, with *S. aurita* exhibiting the highest level (8.59%) and *S. colias* the lowest (0.72%; $p < 0.05$). Consequently, energy values

were highest in *S. aurita* (166.80 kcal/100 g; $p < 0.05$) and lowest in *S. colias* (101.12 kcal/100 g). These interspecific differences reflect species-specific metabolism, activity and habitat: European anchovy and European sardine display seasonal fat shifts linked to plankton availability; mackerel accumulate lipids for migration and round sardinella stores more fat due to its warm-water affinity. Collectively, the results highlight the nutritional relevance of pelagic species as affordable and sustainable contributors to the Mediterranean diet.

The increasing abundance of *S. aurita* in Iberian waters, driven by rising sea-surface temperatures, further underscores its potential as a valuable nutritional alternative to traditional Mediterranean fish resources. The data on protein, fat, moisture and energy enhance food composition databases, support evidence-based dietary recommendations and inform fisheries management by emphasising the role of emerging species such as *S. aurita*. Future research should assess fatty acid profiles across reproductive stages, alongside vitamin D, selenium, and mercury contents.

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Spent coffee grounds-derived water-in-oil-in water double emulsions for edible coffee oil aroma preservation: A sustainable approach

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Spent coffee grounds (SCG) are generated in large quantities by the coffee industry and contain underutilized biomolecules with potential for sustainable food applications. This study focused on the development of water in-oil-in-water (W/O/W) double emulsions, using SCG-derived polysaccharide-rich extracts to protect spent coffee oil (SCO) from oxidation and preserve its aroma.

Double emulsions (DE), particularly water-in-oil-in-water (W/O/W) systems, have gained attention for their ability for enabling the co-encapsulation of lipophilic and hydrophilic compounds within a single structure. This is possible because W/O/W systems provide two distinct aqueous and oil compartments, allowing water- and oil-soluble compounds to be entrapped in separate internal phases while remaining within the same delivery system. Knowing that well-defined double-emulsion droplets are almost exclusively formed with sophisticated microfluidic devices, a simple two-step emulsification process developed using a nonfood-grade oil as model system, has now been successfully used using for the first time a food grade oil.

SCO was recovered by Soxhlet extraction (n-hexane and ethanol), while microwave-assisted extraction of defatted SCG yielded microwave-assisted extracted supernatants (MW1_Sn) and (MW2_Sn), obtained at 150 °C and 180 °C, respectively. Ethanol-recovered SCO exhibited higher phenolic content and antioxidant activity, and both MW extracts displayed similar galactomannan/arabinogalactan-rich profiles, with MW1_Sn containing higher levels of chlorogenic acids. The resulting emulsions exhibited stable droplet morphology, particularly those formulated with MW1_Sn and surfactants., Furthermore, sustaining their aroma protection activity, these emulsions preserved desirable aroma compounds, like furans, while limiting the release of lipid-oxidation markers, as aldehydes. These findings were obtained under sunlight exposure over 37days, showed an enhanced preservation of edible coffee oil (SCO) against photo-oxidative degradation with evidence of short-term stability. Extended storage studies and more detailed physicochemical double emulsions stability assessments will be required to fully evaluate long-term performance. These results demonstrate the feasibility of repurposing SCG into sustainable food-grade emulsions capable of protecting edible coffee oil aroma.[1]

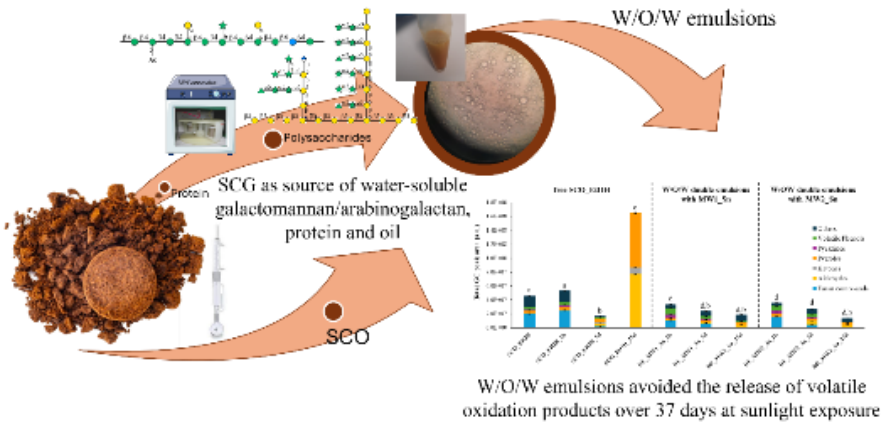


Figure 1 – Integrated process from recovery of oil and polysaccharides to the formation stable water-oil-water double emulsions.

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Wild fruits of Cabinda: Nutritional Composition, Bioactive Properties, and Potential Valorisation

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Wild fruits are an accessible source of food and income for local communities in Cabinda (northern exclave of Angola), where they are well adapted to the prevailing climatic conditions (1). Despite their nutritional value, many native species remain underutilized because of seasonality, high perishability, and inadequate preservation and distribution systems (2). Food preservation technologies, such as dehydration, offer a promising strategy to extend shelf life while preserving nutritional and bioactive composition (3). However, the nutritional and functional properties of these traditional fruits remain poorly characterized. This study evaluated the edible portions of three traditionally consumed fruits in Cabinda (*Spondias dulcis*, *Pachylobus edulis* and *Syzygium malaccense*) after forced-air dehydration. Water activity (a_w), nutritional composition (proteins, lipids, sugars and minerals), total phenolic content (TPC), lipid peroxidation (TBARS) and cytocompatibility were analysed using standardized analytical methods. The dehydrated fruits exhibited a_w values between 0.27 and 0.61, suggesting reduced susceptibility to microbial growth. Protein content ranged from 3.29 to 8.67 g/100 g Dry Weight-DW, lipids from 3.48 to 52.15 g/100 g DW, and total sugars from 0.47 to 46.88 g/100 g DW. Potassium was the predominant mineral (1066.8 to1556.0 mg/100 g DW), followed by calcium (139.2 to 187.4 mg/100 g DW), phosphorus (121.5 to 140.6 mg/100 g DW), magnesium (78.3 to 112.0 mg/100 g DW) and iron (1.4 to 2.3 mg/100 g DW) indicating that these fruits are relevant sources of potassium, calcium and magnesium. *P. edulis* showed the highest lipid content (52.15 ± 0.99 g/100 g DW) and TPC (54.39 ± 0.65 mg gallic acid equivalents -GAE/g DW), whereas *S. malaccense* exhibited the highest potassium (1556 ± 17.1 mg/100 g DW) and total free sugar (46.88 ± 0.86 g/100 g DW) contents. TBARS values ranged from 0.044 to 2.78 mg MDA/kg, indicating differences in lipid oxidation among the fruit species. Preliminary cytocompatibility assays demonstrated that the extracts maintained cell viability above 70% at low and intermediate concentrations in intestinal and hepatic cell models, indicating no cytotoxic effects under the tested conditions. This study provides an integrated characterisation of dehydrated fruits traditionally consumed in Cabinda, a distinct Guineo-Congolian phytogeographical unit with a markedly different floristic composition from most of the country. The findings support their valorisation as nutrient-rich ingredients for the development of local food products.

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Efficacy of controlled atmospheres in inhibiting fungal development and ensuring food safety of chestnuts (Castanea sativa Mill.) during extended storage

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Chestnut (Castanea sativa Mill.) is a fruit of significant economic and cultural importance in northeastern Portugal, particularly in the Trás-os-Montes region, where Protected Designation of Origin varieties such as Judia are widely cultivated. Despite its nutritional richness, postharvest conservation is severely constrained by high moisture content (approximately 50%) and pronounced susceptibility to physicochemical and microbial deterioration. Storage under controlled atmosphere (CA), through modulation of O_2, CO_2 and N_2 concentrations, represents a promising complementary technology to refrigeration for extending shelf life while preserving fruit quality. This study aimed to evaluate the effect of different gaseous compositions on the physicochemical and nutritional quality of Judia chestnuts over a 160 days storage period. The experimental design was based on a Mixture Design with three gaseous components, generating 14 experimental conditions and two controls (positive: conventional refrigeration; negative: ambient atmosphere), each applied in duplicate chambers. Weekly monitoring comprised mass loss, texture profile analysis (TPA), pH, water activity (Aw), and colour parameters (L*, a*, b*) assessed both by instrumental colorimetry (Konica Minolta) and by image processing with Python algorithms, which also enabled automated detection and quantification of fungal and larval contamination from standardised photographic records (DSLR Nikon D5300, 24.2 MP). Nutritional analyses were conducted on freeze-dried samples at 0, 105, and 160 days of storage, encompassing ash, proteins, lipids, fatty acid profile, free sugars, starch, and rancidity, all determined according to AOAC official methods.

Preliminary results reveal a progressive increase in fungal incidence, markedly more pronounced in negative controls, while controlled atmosphere conditions consistently limited microbial development. Mass loss decreased steadily across all CA conditions, and moisture content declined in most treatments, except in negative controls. Biochemical analyses at day 0 indicated an initial characterisation of lipid content, fatty acid profile, free sugars, starch, and rancidity, providing a baseline for comparison with subsequent timepoints at 105 and 160 days of storage. At this stage, general storage conditions appear to exert a dominant influence over the specific gaseous composition, underscoring the need to complete the full 160 days study to discriminate the individual effect of each CA treatment and identify optimal conditions for long term chestnut conservation.

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From Wild Mushroom to Functional Foods: Polysaccharides Characterization and Their Cholesterol-Lowering Potential

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Functional foods are foods that provide health benefits beyond basic nutrition, due to the presence of bioactive compounds that contribute to disease prevention. Mushrooms are recognized as functional foods due to their chemical composition, particularly their polysaccharides content, which have been associated with cholesterol-lowering activity. Several *in vitro* and *in vivo* studies have demonstrated the cholesterol-lowering effects of mushrooms powder and its polysaccharides-rich extracts, however, the relationships between polysaccharides chemical characteristics and these effects remain unclear[1]. This study aimed to characterize the polysaccharides present in three highly valued wild edible mushroom species, native to Northeast Portugal and associated with the Mediterranean diet – *Lactarius deliciosus*, *Macrolepiota procera* and *Fistulina hepatica*. Also, the cholesterol-lowering effects of fractions rich in these polysaccharides were evaluated using a simplified in vitro intestinal model[2].

Fruiting bodies of *L. deliciosus, M. procera* and *F. hepatica* were extracted with hot water and the aqueous extracts were fractionated with graded ethanol precipitation. Water-insoluble residue and water-soluble rich fractions were chemically characterized by glycosidic linkages analysis of the partially methylated alditol acetates by GC-MS. All mushroom's water-insoluble polysaccharides were composed of glucose with the presence of t-Glc (10 – 17 % mol), (1→3)-Glc (15 – 26 % mol), (1→4)-Glc (2 – 39 % mol), (1→6)-Glc (4 – 13 % mol) and (1→3,6)-Glc (10 – 14 % mol) linkages. The water-soluble fractions were composed of galactans and glucans, with the presence of T-Gal, (1→6)-Gal, (1→2,6)-Gal, T-Glc, 3-Glc, 6-Glc, and 3,6-Glc linkages. Glycosidic linkages analysis revealed the presence of a rhamnogalactan and fucogalctan, confirmed by the occurrence of T-Rha and T-Fuc residue in water-soluble fraction of *L. deliciosus* and *M. procera* or *F. hepatica*, respectively. The 4-Glc and 4,6-Glc residues, identified in some fractions, are indicative of the presence of glycogen, which was subsequently hydrolyzed with α-amylase.

The cholesterol-lowering activity of both water-insoluble and water-soluble polysaccharides-rich fractions was evaluated. Cholesterol solubility was determined via GC-MS, using TMS-cholesterol derivatives, while bile salt binding capacity was measured via ^{1}H NMR, using the CH_3 (C-18) resonance of bile salts present in micellar solution. The rhamnogalactan-rich fractions showed higher cholesterol decrease (50 – 90%) than fucogalactan-rich fractions (13 – 56%) and water-insoluble glucans (13 – 34%). The bile salts sequestration results were similar independently of the polysaccharide (0 – 38%).

The water-soluble polysaccharides present in *L. deliciosus*, *M. procera* and *F. hepatica* exhibited significant effects on cholesterol solubility. Chemical characteristics, such as the presence of glucans covalent linked to the galactan, the presence of terminally-linked rhamnose or fucose residue in galactans, and the polymerization degree appears to influence cholesterol solubility through hydrophobic interactions, suggesting that cholesterol may also directly interact with the polysaccharides. These results highlight the complex role of mushrooms polysaccharides in cholesterol absorption in intestine.

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Mushrooms and Their Potential as Sustainable Biosolutions

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Endogenous forest resources have been assuming an increasingly prominent role in the sustainable valorisation of natural raw materials, with wild mushrooms standing out as a promising source of bioactive compounds and natural pigments. Embedded in ecosystems of high biodiversity, mushrooms present a rich composition of nutrients, including vitamins, minerals, proteins and polysaccharides, as well as diverse molecules with bioactive properties, such as antioxidant and anti-inflammatory compounds. Beyond their nutritional value and pharmacological potential, wild mushrooms contain natural pigments that can be exploited across different applications, namely in the food, pharmaceutical, cosmetic and textile industries. These pigments, frequently associated with phenolic compounds and other secondary metabolites, contribute not only to coloration but also to functional properties such as antioxidant activity, reinforcing their interest as natural alternatives to synthetic dyes. In Portugal, more than 2,000 mushroom species have been identified, with mountainous and humid regions being the most conducive to their occurrence.
The controlled and guided harvesting of these species thus emerges not only as a food safety measure, but also as a strategy for valorising their compounds, namely the pigments that constitute part of the fungi's own chemical defence mechanisms promoting their industrial application within the scope of biopigment production.[2]

Each species may exhibit distinct colourations, as fungi develop unique metabolic pathways for the synthesis of complex pigments, including carotenoids, anthraquinones, terphenylquinones and betalains.[2] The extraction of these natural pigments can range from simple hot water decoction to more advanced methodologies, such as ultrasound-assisted extraction or the use of organic solvents with pH adjustment, always framed within the principles of green chemistry and environmental sustainability.[3]

In this context, the BioDyes project proposes an innovative biotechnological approach for the production, isolation and purification of biopigments obtained through bacterial and fungal metabolic processes, aiming at a broad colour palette based on the CMYK system for the dyeing and printing of natural and synthetic fibres. Led by Adalberto Textile Solutions, S.A., and developed in partnership with four entities of the Research and Innovation System IMM, INIAV, FAULisboa and UMinho with 2C2T the project seeks to achieve reproducibility and industrial scalability, with a direct impact on reducing industrial effluent contamination and without risks to human health. This initiative is framed within a circular economy and sustainable development logic, promoting the use of local resources, biotechnological innovation and the creation of new value-added products.

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FT-NIR Authentication and Differentiation of Golden Delicious "Alcobaça" Apple

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The geographical indication "Alcobaça" Apple covers various varieties, with Golden Delicious being among the most popular. Certified fruits have a thin, juicy and consistently pleasant pulp that is slightly acidic and well-balanced in aroma. The production areas are confined to the municipalities of "Alcobaça", "Nazaré", "Caldas da Raínha", "Óbidos" and "Porto de Mós".

The presence of this Protected Geographical Indication (PGI) region enhances the commercial value of these apples. Consequently, there is interest in developing methods to characterise and distinguish apples from different IGP regions. In this study, Golden Delicious apples were collected from various IGP orchards and from an orchard outside the designated area. Several physical, chemical, and nutritional analyses were performed, including total soluble solids, acidity, vitamin C, pH, total phenols, colour, fat, fibre, protein and ash. FT-NIR spectroscopy was used to acquire spectra with a resolution of 8 cm^{-1} and 32 scans in reflectance mode, covering the range from 16 000 to 4 000 cm^{-1} using apple pulp.

Apples with IGP had a higher total phenolic content (762-1,251 mg/kg) than the apples without IGP certification (386-487 mg/kg). The samples with IGP also exhibited higher levels of ascorbic acid and differences in soluble solids content, indicating clear compositional differentiation between the two groups.

The same separation seen in Figure 1A was also evident when PCA was performed using spectral data from FT-NIR (Figure 1B).

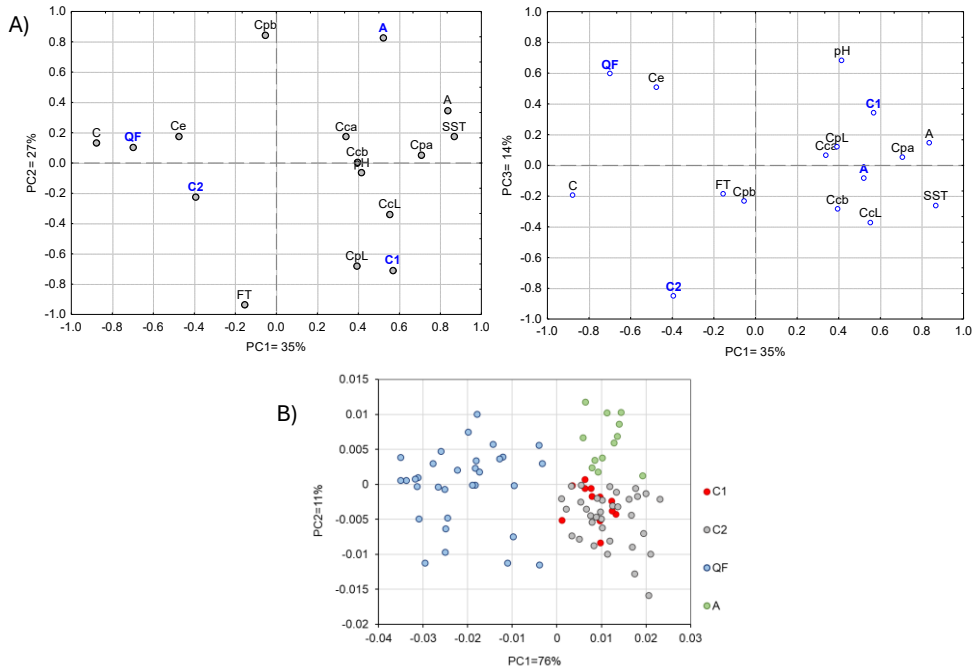


Figure 1 - Principal component analysis based on analytical parameters (A- PC1 vs PC2 and PC1 vs PC3) and in spectral information (B). C: Ashes; A: Acidity; VC: Vitamin C; SST: Total Soluble Solids; CpL: Colour pulp L *; Cpa: Colour pulp a *; Cpb: Colour pulp b *; CcL: Shell colour L *; Cca: Shell colour a *; Ccb: Shell colour b *; C1 - IGP apples from Centieiras Orchard 1; C2 - IGP apples from Centieiras Orchard 2; QF - IGP apples from Quinta da Feteira; A - non IGP apples from Armamar.

Principal component analysis, based on analytical parameters, explained 76% of the total variance (PC1 = 35%, PC2 = 27% and PC3 = 14%). The score plots showed partial differentiation between orchards related to variables such as pH, colour parameters and phenolic compounds. The PCA of the NIR spectra explained 87% of the total variance (PC1 = 76%, PC2 = 11%) and revealed a clearer clustering pattern, with the QF samples clearly separated from the other groups. Apples

produced outside the IGP area formed a distinct cluster with limited overlap. These results highlight the potential of FT-NIR spectroscopy to characterise apple samples according to their origin, even within the same production region.

It is important to note that FT-NIR spectra can be affected by physical characteristics such as firmness and water content, which can vary between orchards due to environmental conditions and agronomic practices. For this reason, the PCA separation based on physicochemical parameters and FT-NIR is not identical.

This approach appears promising for authenticating apples with IGP and from different regions. However, it is important to recognise that IGP orchards exhibit considerable variability in quality characteristics, even within IGP standards.

Therefore, these findings should be validated using a larger sample from various orchards, both inside and outside the IGP.

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Physicochemical and textural evaluation of wheat bread enriched with Ulva sp., *Himanthalia elongata* and *Palmaria palmata*

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Bread remains a popular food worldwide with a huge source of energy and essential nutrients in the human diet, making it an excellent matrix for nutritional enrichment [1]. The incorporation of edible macroalgae has emerged as a strategy to improve the nutritional value of bread due to their content in, essential minerals, dietary fiber and bioactive compounds [2, 3]. However, the high water-holding capacity of seaweeds represents an important technological challenge during dough processing. This study aimed to evaluate the effect of incorporating three edible macroalgae species *Ulva sp.*, (green), *Himanthalia elongata* (brown) and *Palmaria palmata* (red), into wheat bread while optimizing dough hydration according to the water absorption capacity and intrinsic salt content of each species.

Ten bread formulations were produced, including one control and nine breads containing 2, 4 and 6% macroalgae (w/w, flour replacement). The seaweeds were dried, milled and standardized to a uniform particle size before incorporation. Dough formulations were adjusted to compensate for the different water-holding capacities and natural salt contents of each macroalgae, ensuring comparable dough consistency.

Bread samples were characterized for moisture content, water activity, color evaluation (CIELab) and Texture Profile Analysis. Texture was performed on Day 0 (after cooling to room temperature and thermal stabilization), and after 24 and 48 h of storage to evaluate changes associated with bread staling.

No significant differences in whole-bread hardness were observed between the control and macroalgae-enriched formulations throughout storage. However, crumb analysis revealed that breads containing *Ulva sp.* and *Palmaria palmata* improved gumminess retention during storage, while elasticity remained comparable among all formulations. These preliminary results suggest that hydration-adjusted incorporation of macroalgae allows enrichment of wheat bread without compromising its technological quality and may contribute to improved crumb stability during storage. The hydration adjustment strategy based on the determined water-holding capacity of each macroalgae enabled the production of doughs with comparable handling properties despite the different incorporation levels.

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Socioeconomic Drivers of Traditional Plant Knowledge and Their Relevance to Food Heritage in the Natural Park of Serra da Estrela

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Traditional plant knowledge is a key component of community health, local identity, and biocultural heritage, particularly in mountain regions where plant-based preparations are deeply embedded in daily life. In Serra da Estrela Natural Park, Portugal, a protected mountain landscape marked by strong ecological and cultural diversity, several traditional practices involve orally consumed preparations, especially infusions, linking medicinal plant knowledge to a broader food–remedy continuum. However, the distribution of this knowledge within local populations is uneven, and mountain protected areas in Europe remain insufficiently studied in this regard[1-3]. This study aimed to quantify intracultural variation in medicinal-plant knowledge within the Serra da Estrela Natural Park and to assess its association with socio-demographic and geographic factors, while discussing its relevance for safeguarding traditional food-related heritage.

A cross-sectional ethnobotanical survey was conducted with 286 informants across five municipalities of Serra da Estrela. Semi-structured interviews documented 133 medicinal plant species, including native, introduced, cultivated and commercially available plants, and recorded information on plant uses, plant parts, preparation methods, routes of administration and associated disease categories. Particular attention was given to domestic plant-based preparations, because they reflect the everyday interface between food, care and local memory. Data were analysed using non-metric multidimensional scaling (NMDS), permutational multivariate analysis of variance (PERMANOVA), and generalised linear models (GLMs). A network approach was also used to summarise the links between frequently cited plant families and disease categories [1,2].

The survey documented a rich and socially structured body of traditional knowledge, with 4899 use reports distributed across 107 ailment categories. Native species accounted for a significant proportion of the recorded flora, while cultivated and commercially available plants showed how local knowledge adapts to changing access to plant resources. Herbs were the dominant growth form, while leaves and flowers were among the most frequently used plant parts. Oral administration was the primary route of use, and infusions were the most common preparation method, underscoring their role as accessible household practices situated between food and remedy. Knowledge composition and richness varied significantly among social groups. Women contributed 3507 use reports, compared with 1392 reported by men, confirming their central role in the preservation and transmission of traditional plant knowledge. PERMANOVA showed non-random differences according to marital status ($F = 36.55$, $p = 0.0001$; Bonferroni pairwise $p = 0.0006$). GLMs identified both geographic and social predictors of knowledge richness. For digestive uses, Celorico da Beira ($B = -0.302$, 95% CI −0.512 to −0.091, $p = 0.005$) and Guarda ($B = -0.540$, 95% CI −0.755 to −0.325, $p < 0.001$) listed fewer species than Gouveia. For respiratory uses, several municipalities listed more species than Gouveia, including Seia ($B = 0.769$, $p < 0.001$). Singles also reported fewer genitourinary symptoms than widows ($B = -0.478$, $p = 0.039$). These findings show that traditional knowledge is not uniformly distributed but is structured by municipality and social factors [1].

The documented plant-based practices, particularly infusions and other domestic preparations, represent more than therapeutic knowledge: they are part of a living heritage shaped by biodiversity, household practices, gender roles, ageing, municipality, and rural identity. The network analysis further indicates that frequently cited plant families are connected with major use categories, suggesting that local communities organise plant knowledge through both therapeutic experience and everyday cultural practice. By identifying who holds this knowledge and how it varies across communities, this study provides decision-relevant evidence to safeguard biocultural heritage and to integrate local knowledge into conservation, rural health planning, and food heritage valorisation strategies. In the context of traditional foods, these results support the recognition of plant-based preparations as culturally embedded practices that connect food, remedy, memory, and sustainable use of local resources.

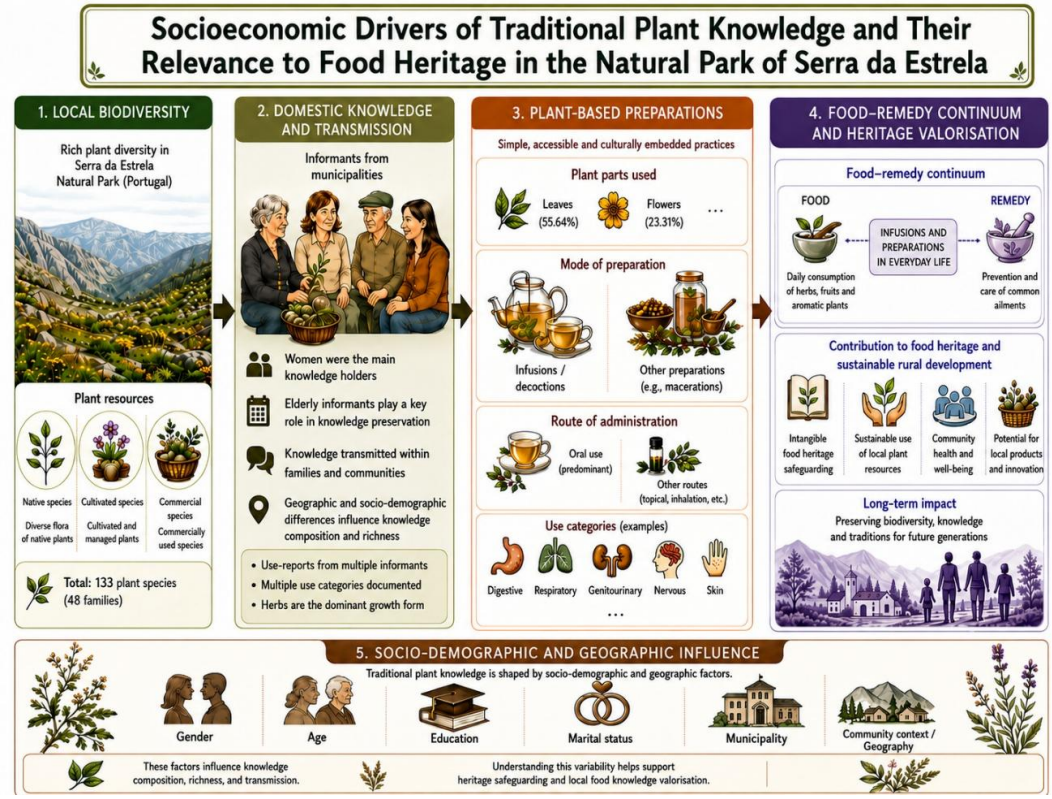


Figure 1. Conceptual overview of the study on traditional plant knowledge in the Natural Park of Serra da Estrela and its relevance to food heritage.

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Unlocking the Bioactive Potential of Beira Interior Red Fruits for Metabolically Health-Oriented Food Supplements

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The valorisation of local red fruits from Beira Interior represents a promising strategy for developing functional foods and dietary supplements targeting metabolic syndrome[1]. These endogenous products, including cranberry, blueberry, elderberry, cherry, and peach, are of particular interest due to their rich content of bioactive compounds and their potential antioxidant and antidiabetic properties[2]. This work aimed to conduct a preliminary biological evaluation of industrial- and laboratory-prepared 70% hydroethanolic red fruit extracts to support the selection of the most promising matrices for future supplement prototyping (Figure 1). The tested samples included 70% hydroethanolic extracts of industrial cranberry (C-IND), industrial blueberry (M-IND), industrial elderberry (S-IND), laboratory-prepared elderberry (S-LAB), laboratory-prepared peach (P-LAB), laboratory-prepared cherry (CH-LAB), and laboratory-prepared blueberry (M-LAB). Extract mixtures were also evaluated, including cranberry/elderberry (CS), blueberry/elderberry (MS), blueberry/cranberry (MC), and blueberry/cranberry/elderberry (MCS).

The biological workflow included cell-free DPPH•, superoxide (SO_{-2}•) and nitric oxide (NO•) radical scavenging assays, an α-glucosidase inhibition assay, and cytotoxicity assays (MTT/LDH) in normal human dermal fibroblasts (NHDF). This strategy is aligned with the project objective of characterising raw materials and selecting the most promising ingredients for future food supplement development. In the DPPH•assay, C-IND showed stronger antioxidant activity than S-IND, whereas in the superoxide (SO_{-2}•) assay, M-IND showed the best activity among the tested fruit extracts, followed by S-IND and C-IND. In the NO• scavenging assay, C-IND showed the strongest activity among the evaluated fruit extracts, with a mean IC_{50} of 97.383 µg/mL, followed by S-LAB (138.154 µg/mL), S-IND (207.531 µg/mL) and M-IND (562.356 µg/mL). For P-LAB, the reported endpoint was IC_{25}, with a mean value of 630.758 µg/mL. In the α-glucosidase inhibition assay, C-IND showed the most promising profile, with an IC_{50} of 68.677 ± 4.601 µg/mL, lower than that of acarbose (334.688 ± 11.250 µg/mL) under the same experimental conditions. S-LAB and S-IND exhibited weaker α-glucosidase inhibitory activity, with IC_{50} values of 1324.080 ± 138.036 µg/mL and 1686.510 ± 57.914 µg/mL, respectively. P-LAB was expressed as IC_{25}, with a value of 2361.543 ± 272.464 µg/mL. Cytotoxicity was assessed in NHDF cells using MTT and LDH assays. Among the industrial extracts, C-IND showed the highest IC_{50} value in NHDF cells (589.9 ± 31.2 µg/mL), suggesting the best cellular tolerance, followed by S-IND (418.7 ± 19.2 µg/mL) and M-IND (283.8 ± 19.3 µg/mL). The cytotoxicity of extract mixtures was also evaluated. CS showed the highest IC_{50} value, 786.4 ± 48.7 µg/mL, suggesting the best cellular tolerance among the tested combinations. It was followed by MCS (696.75 ± 63.15 µg/mL), MC (548.3 ± 114.0 µg/mL), and MS (421.4 ± 35.9 µg/mL). These results indicate that CS and MCS may be the most suitable mixtures for further biological evaluation, whereas MS should be interpreted with caution, given its lower IC_{50} value.

These preliminary findings suggest that C-IND, corresponding to the industrial 70% hydroethanolic cranberry extract, is the most promising sample among the tested matrices, combining NO radical scavenging and α-glucosidase inhibitory activities with favourable cellular tolerance in NHDF cells. The CS and MCS mixtures also appear relevant for subsequent formulation-oriented studies. This integrated approach will support the selection of the best-performing red fruit ingredients for pilot-scale development of functional food supplements.

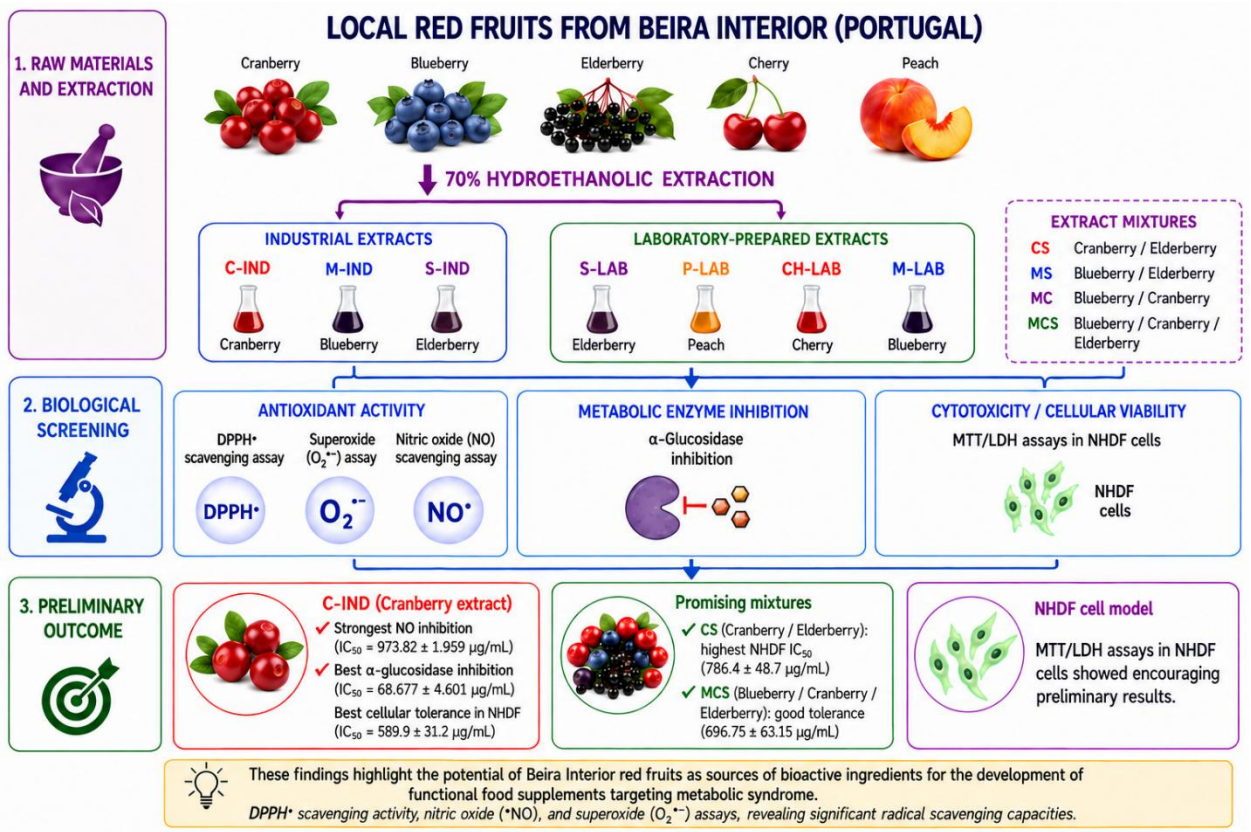


Figure 1. Integrated biological screening workflow used to evaluate local Beira Interior 70% hydroethanolic red fruit extracts and select the most promising candidates for functional food supplement development.

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Phenolic Composition vs. Pollen Profile of Terra Quente PDO Honey

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Phenolic compounds are among the most important bioactive constituents of honey, contributing to its antioxidant properties and serving as valuable indicators of quality, authenticity and botanical origin. In this study, the phenolic compounds and palynological profile of honey samples with Protected Designation of Origin (PDO) from the Terra Quente region were evaluated. A total of 22 honey samples produced during 2024 were analysed. Phenolic compounds were extracted and analysed by high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD), allowing their separation, identification and quantification. Eighteen phenolic compounds were detected, with epicatechin, phloridzin and ellagic acid among the most frequently identified compound. These compounds mainly belonged to the groups of phenolic acids and flavonoids, with concentrations ranging from [1.59-18.97 µg/g], [0.57-2.58 µg/g] and [0.67-0.94 µg/g] respectively. Palynological analysis, performed using the acetolysis method, supported the botanical origin of the samples and indicated a predominantly multifloral profile, with Cistus spp., Lavandula spp., Echium spp., Rubus spp. and Cytisus spp. as the main pollen types.

Overall, the phenolic profiles revealed considerable variability among samples, reflecting the influence of floral and environmental factors characteristic of the Terra Quente region.

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Integral Utilization of Amazon River Prawn (Macrobrachium amazonicum) as a Strategy to Enhance the Food Heritage of Communities in the Amapá Amazon, Brazil

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The Amazon River prawn (Macrobrachium amazonicum) is an important fishery resource for traditional Amazonian communities, contributing to food security, income generation, and the maintenance of regional food practices. However, prawn processing can generate around 50% waste[1], consisting of structures such as the cephalothorax and exoskeleton, a significant portion of the biomass of this crustacean that is not utilized. In this context, full utilization of the prawn represents a sustainable alternative to reduce waste, add value to artisanal fishery products, and enhance traditional foods. Thus, the present study aimed to develop food products from the full utilization of the Amazon River prawn, evaluating its nutritional potential and applicability as an alternative for valorizing fishery resources used by communities in the Amapá Amazon.

For product development, the raw material was purchased at a market in the city of Macapá, Amapá, Brazil, and subsequently transported to the Fish Biology and Fish Processing Laboratory of the Amapá State University. The prawns were manually peeled to obtain the muscle. The processing residues, cephalothorax and exoskeleton, were used to produce prawn flour and a snack called "little spider" of prawn (Figure 1), aiming at the full utilization of the different parts of the crustacean. The yield of the obtained products was evaluated, and physicochemical analyses were performed, including moisture, ash, protein, lipids, carbohydrates[2], caloric value, pH, and water activity (aw).



Figure 1 A – Specimens of Macrobrachium amazonicum. B – Preparation of the snack "little spider" of prawn. C – Processing residues of Macrobrachium amazonicum. D – Vacuum-packed flour produced from processing residues of Macrobrachium amazonicum.

A total of 6.865 kg of whole prawn was used, from which 3 kg of muscle were obtained, representing a yield of 43.69%. From the prawn processing residues (3.865 kg), it was possible to produce 0.750 kg of the "little spider" of prawn snack, while the remaining material was used for flour production, resulting in 0.580 kg after the drying process, with yields of 19.40% and 18.61%, respectively, highlighting the potential use of parts that are traditionally discarded. The overall yield was 81.70%, with the remaining portion corresponding to water loss during flour processing. In this sense, full utilization was achieved through the development of coproducts from the processing residues.

The determination of fish chemical composition allows their classification into major food groups according to water, lipid, protein, and mineral contents.[3] Thus, the analysis of the main components of a food product is important and necessary, among other reasons, for product standardization, evaluation of changes in chemical composition, and determination of dietary characteristics. The products developed from Amazon River prawn processing residues showed high nutritional value, particularly in terms of protein content, with 41.90% in the flour and 15.28% in the "little spider" of prawn (Table 1). These results confirm the potential of these products for human consumption, either as an ingredient for enriching low-protein foods or as an alternative snack option.

Table 1 - Physicochemical composition (mean ± standard deviation) of the muscle, prawn waste flour, and the "little spider" of prawn snack of Amazon River prawn (*Macrobrachium amazonicum*).

Parameters	Muscle	Prawn flour	"little spider" of prawn
Moisture (%)	81.54±1.34	4.51±0.17	73.56±0.16
Ash (%)	1.03±0.11	30.25±0.81	6.58±0.16
Total lipids (%)	0.13±0.06	8.31±0.73	1.33±0.15
Total proteins (%)	18.23±0.87	41.90±0.91	15.28±0.32
Carbohydrate (%)	0±2.13	15.12±1.97	3.17±0.44
Caloric value (kcal/100 g)	72.3±5.30	311.5±4.53	87±0.40
pH	7.61±0.01	9.60±0.06	9.16±0.02
Water activity (aW)	0.90±0.001	0.46±0.056	0.90±0.001

The full utilization of the Amazon River prawn presents potential as a strategy for valuing the Amazonian food heritage, transforming commonly discarded parts into nutritionally valuable products using low-cost, low-technology approaches. This approach may contribute to the sustainability of the fish production chain, strengthening traditional practices of fishing communities in the Amapá Amazon, promoting income generation, reducing environmental impacts, and enhancing the value of regional food resources.

In addition, the knowledge generated on the full utilization of the prawn may be transferred and directly applied in communities through artisanal courses and training activities, enabling local production of these products and encouraging community-based entrepreneurship, food security, and sustainable development.

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The evolution of aged WS during consumption: its relationship with ageing technologies

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Wine spirit (WS) is an alcoholic beverage that is usually consumed after being aged in wooden barrels for a minimum of six months[1]. Current research on this beverage focuses on alternative technologies aimed at increasing sustainability by reducing environmental impacts, while improving economic and social outcomes[2]. In this context, the research project OXYREBRAND (https://projects.iniav.pt/oxyrebrand/index.php/pt/) was developed to study aged WS resulting from different ageing technologies (traditional technology using wooden barrels, and alternative technology combining wood staves with different levels of micro-oxygenation – MOX, and nitrogen as a control to assess the influence of oxygen during ageing), using the same wood botanical species, the Portuguese chestnut (Castanea sativa Mill.). To this end, the same "Lourinhã" wine distillate was distributed by 250 L barrels, and by 50 L glass demijohns with chestnut wood staves inside combined with MOX (with different flow rate), in duplicate, and it was aged for 12 months[2]. In this research project the knowledge on the role of oxygen and the influence of oxidation reactions occurring during ageing has been deepened. As a result, evidence exists on the influence of MOX level on the evolution of the aged WS during storage in bottle[2]. However, to the best of our knowledge, it remains unknown what happens during its consumption. It is therefore imperative to understand if its physicochemical characteristics and sensory properties, imparted by the ageing technology remain after opening the bottle and during its consumption, maintaining the quality attributes. Since quality attributes are evaluated over time, it can be inferred that we are assessing the beverage's shelf life (SL). The SL of a food or drink is, by definition, the period during which the product retains its microbiological safety and sensory characteristics under specified storage conditions[3]. Due to its high ethanol content and low pH, aged WS is considered a microbiologically stable alcoholic beverage. However, to the best of our knowledge, no specific SL has been established for this product. The aim of the present work was to determine whether the SL of WS is influenced by the ageing technology used. Thus, the following physicochemical characteristics — acidity (total and volatile), total dry extract and pH — were analysed using OIV methods, as well as the total polyphenol index (TPI)[2], in duplicate, at 7 and 11 months after opening the bottle. The results revealed that WS samples aged under conditions that limited oxygen exposure contact during ageing tended to exhibit a shorterSL that those produced using other ageing technologies. This behaviour was evidenced by their lower total and volatile acidity, total dry extract and TPI, together with higher pH value. No significant differences were detected among the ageing modalities employing MOX, suggesting that differences in oxygen flow during ageing do not affect the SL. Conversely, the traditional ageing technology showed a tendency to produce WS with a longer SL. In summary, these initial results suggest that the SL is influenced by the ageing technology.

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Anthocyanin-Mediated Formation of Strecker Aldehydes in Model Wine Solutions: Identification of Potential Precursors from Malvidin-3-O-Glucoside and Delphinidin-3-O-Glucoside

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Anthocyanins are key contributors to the colour of young red wines but undergo extensive chemical reactivity during ageing, including degradation and condensation reactions that lead to the formation of new pigments[1] and aroma-active compounds[2]. Strecker aldehydes are major contributors to the characteristic oxidative aroma of Tawny Port wine. A recent study[2] demonstrated that different anthocyanins display distinct efficiencies in promoting the formation of Strecker aldehydes in model wine systems, with malvidin-3-O-glucoside (oenin) showing higher activity than delphinidin-3-Oglucoside (myrtillin). This observation is unexpected, as Strecker-related oxidative processes are commonly associated with phenolic structures containing o-catechol groups. These findings suggest that Strecker aldehyde formation may depend on specific oxidative degradation pathways of anthocyanins rather than solely on their initial hydroxylation pattern.

In this work, model wine solutions (pH 4.2) containing mixtures of amino acids (L-leucine and L-phenylalanine) and iron as a catalyst were prepared with either oenin or myrtillin. The mixtures were incubated at 37 °C for approximately 30 days and monitored weekly by HPLC-DAD. At the end of the incubation period, the solutions were analysed by solid-phase microextraction (SPME) coupled to GC-MS to determine volatile compounds formed during the reaction, focusing on Strecker aldehydes. The solutions were also analysed by HPLC-DAD-MS/MS to identify potential intermediates formed during anthocyanin degradation that could promote Strecker aldehyde formation.

Comparison of the obtained results aims to explain the unexpectedly higher contribution of oenin, compared with myrtillin, to the formation of Strecker aldehydes in wine model solutions.

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From rind to strain bank: biochemical and molecular identification and the strategic value of preserving *Pseudomonas* isolates from traditional Portuguese cheeses

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The maintenance of strains isolated from the rind of Castelo Branco and Serra da Estrela cheeses in a culture collection is of particular scientific and technological relevance, in light of the results obtained in the present study. The 29 isolates, initially characterised as Gram-negative, oxidase-positive, glucose-fermenting and, in many cases, fluorescent under UV light, exhibited a phenotypic profile compatible with the genus Pseudomonas. Complementary assays, namely growth at 6.5 °C, reinforced this presumption, allowing for robust phenotypic screening prior to molecular identification[1].

The comparison between biochemical identification by API 20 NE and molecular identification by 16S rRNA gene sequencing revealed limited agreement. Only three strains showed coincident identification as Pseudomonas fluorescens for both methods highlighting the taxonomic complexity of the genus Pseudomonas and reinforcing the need for polyphasic approaches, combining phenotypic and genotypic methods, for reliable microbial identification[2].

In this context strain cryopreservation in a culture collection assumes a central role. Firstly, it ensures the preservation of autochthonous microbial biodiversity associated with traditional Portuguese cheeses, a biological heritage of considerable value for microbial ecology studies and characterisation of microbiota specific to Protected Designation of Origin (PDO) regional products. Secondly, it enables the design of reproducible assays, essential for investigating the role of these bacteria in ripening processes, in the formation of aroma compounds, in pigmentation phenomena, or in spoilage mechanisms. Additionally, the continued availability of these strains enables the development of future research lines, including genomic studies, microbial interaction assays and potential biotechnological applications [3].

In summary, the present study demonstrates not only the importance of combining biochemical and molecular methodologies for the identification of environmental isolates, but also the strategic value of maintaining these strains in a culture collection, ensuring their long-term scientific and technological use.

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Evolution of Quality Attributes and Free Amino Acids during Extended Beef Ageing

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Dry ageing is widely recognised for improving beef sensory quality through biochemical and structural changes occurring during storage. This process involves storing beef cuts under strictly controlled environmental conditions, specifically temperature, relative humidity, and airflow, without protective packaging for several weeks. During this period, endogenous proteolytic enzymes break down muscle fibres, significantly enhancing tenderness[1,2].

This study evaluated the effect of a 60-day ageing period on physicochemical characteristics, shear force/tenderness and free amino acid (FAA) profile of two beef cuts, chuck and sirloin.

Four chucks and four sirloins obtained from four Holstein–Friesian cattle were used in the study. Each cut was subdivided into four portions corresponding to ageing periods of 20, 30, 45 and 60 days, resulting in a total of 32 samples. Physicochemical parameters included pH, intramuscular fat content, cooking losses, instrumental colour (L*, a*, b* and chroma) before and after blooming, and shear force. Free amino acids were quantified to assess proteolytic activity during ageing[3].

Ageing significantly affected pH ($P < 0.001$), while cut type had no significant influence. Fat content was higher in chuck than in sirloin ($P = 0.012$), whereas cooking losses remained unchanged throughout ageing. Colour parameters were generally stable, with no significant changes in redness (a*), indicating preservation of visual quality.

The shear force was the parameter most affected by ageing, with highly significant effects of the cut ($P < 0.001$), time ($P < 0.001$) and their interaction ($P < 0.001$). In the chuck, a marked reduction in shear force was observed between 20 and 30 days, from 4.52 kg to 2.67 kg, indicating a substantial improvement in tenderness in the early stages of maturation. Subsequently, values increased, although they remained lower than those initially recorded. In the sirloin, the evolution showed a different behaviour.

The values decreased progressively throughout the experimental period, reaching the minimum value at 60 days (2.81 kg). This result suggests that this muscle continues to benefit from longer maturation periods. The improvement in tenderness observed can be explained by the degradation of myofibrillar proteins promoted by endogenous enzymes, particularly calpains. The differentiated response between muscles could be related to differences in connective tissue composition, muscle structure, and the specific proteolytic activity of each muscle group.

The FAA profile revealed intense proteolytic activity during ageing. Total FAA increased from 271.5 to 1714.2 µg/100 g in sirloin and from 694.9 to 1973.8 µg/100 g in chuck, although a decline was observed in chuck at the final ageing stage. Substantial increases were detected in glutamic acid, aspartic acid, glycine, threonine, histidine, lysine and branched-chain amino acids, compounds known to contribute to umami taste and to act as precursors of aroma compounds generated during cooking.

Overall, the results demonstrate that prolonged ageing promotes significant proteolysis and improves tenderness while maintaining colour stability. The accumulation of free amino acids suggests enhanced potential for flavour development, highlighting the importance of ageing time and muscle type in determining beef quality.

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Rediscovering Portuguese biodiversity: Valorization of endogenous berries through pasta enrichment

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Food extends beyond its essential role in sustaining human life and is recognized as a key element of cultural heritage where elements of food culture such as traditions, knowledge, values, identity, production methods, ingredients, products, culinary practices, and symbolisms are preserved and transmitted throughout generations[1,2]. As such, food serves as an important vehicle for cultural expression, social cohesion, and the preservation of collective memory. Endogenous berries are recognized as important sources of nutrients and bioactive compounds, with health benefits and relevant applications in food products, additives and nutraceuticals. Additionally, the consumption of wild berries promotes the conservation of agrobiodiversity, aligns with sustainable food systems and promotes the preservation of food heritage[3].

This study aimed to use pasta, a widely consumed, shelf-stable, affordable, and convenient food product, as a vehicle for the incorporation of an endogenous berry (EB). The objective was to enhance the nutritional profile of the final product while preserving its accessibility, ease of preparation, and storage stability. The fruits pulp was dried in a drying chamber at 40 °C for 24 h, and subsequently, milled into powder. Pasta samples were formulated using either wheat flour or buckwheat flour, eggs, olive oil, and enriched with 0%, 2.5% or 5% of dried EB, and molded into pasta strips. Both fresh and dehydrated pasta samples were characterized in terms of cooking process, texture, dimensions, color and rheology. The dehydration process consisted of placing the pasta samples in a drying chamber at 40 °C for approximately 12 h.

The optimal cooking time varied from 4 min 13 s to 11 min 13 s, with fresh pastas requiring less time to reach optimal cooking time than the dried formulations. The results also indicate that buckwheat pastas have a shorter optimal cooking time than wheat-based pastas. The cooking yield ranged from 154.4% to 177.0% for fresh pastas and 176.3% to 275.6% for the dried counterparts. The dimensional characteristics, width and thickness, showed an increase after cooking in all pasta types - fresh and dried, prepared with wheat or buckwheat. In terms of color, it was observed that, for both wheat and buckwheat cooked pastas, increasing the percentage of fruit to 5% reduced the L* color coordinate (lightness) and the b* coordinate (yellowness) and increased the a*coordinate (redness). These results are consistent with the natural red-purple pigments of the EB, which contribute to an increase of the redness appearance of both pastas. Textural data indicates that pasta with formulations containing 2.5% EB have firmness values more similar to those of the control samples, both for wheat and buckwheat-based pastas. For both types, firmness increased after cooking, indicating that the samples gain integrity and increase cohesiveness during the cooking process. The dried cooked samples with 2.5% EB, regardless of having wheat or buckwheat, showed values of stickiness close to those of pasta formulations with no EB, revealing that the incorporation of this additive affects the samples' stickiness. Furthermore, the results for adhesives showed a similar trend to that observed for stickiness with the samples containing 2.5% EB for both wheat and buckwheat-based formulations.

Moreover, the pasting properties of the pasta have shown that the increased percentage of incorporation of EB had no pronounce alterations in the pasting temperature of buckwheat samples. For peak time, in buckwheat-based pastas, a trend was observed in fresh pastas with the incorporation of EB reducing the peak time, compared to the control samples. In contrast, for wheat-based pastas, no pronounced changes in peak time were observed with the incorporation of the EB. The breakdown viscosity decreased as the EB ratio increased in all pasta samples, with the exception of the wheat-based fresh pasta.

In conclusion, wheat-based pastas exhibit greater consistency than those made with buckwheat, which presented greater shaping and cooking difficulties, as wheat flour possesses superior dough-forming properties due to its gluten content, making it more suitable for pasta production than buckwheat flour. Nevertheless, based on the results, the incorporation of EB powder in pasta is a promising approach for the valorization of this endogenous food product, providing a strategy to enhance physicochemical properties and potentially incorporate bioactive compounds into a commonly consumed food product.

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Funding: This work was financially supported by CICECO Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025, financed by national funds through the FCT/MCTES (PIDDAC) and the Research Centre for Natural Resources, Environment and Society - CERNAS (UIDB/00681; DOI: 10.54499/UIDP/00681/2020).

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Effect of Enzymatic Soaking on the Bioactive Composition of Cooked Brown Rice

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Bioprocessing strategies are increasingly applied to improve the technological, nutritional and functional quality of rice-based products[1,2]. Among these, soaking combined with enzymatic treatment has been proposed to soften cooked brown rice by modifying the outer grain layers, thereby improving water uptake and reducing cooking time. However, soaking may also promote the loss or redistribution of soluble bioactive compounds.

This study evaluated the effect of enzymatic soaking on the bioactive composition of cooked brown rice. Brown rice was soaked for 18 h in water (control) or in solutions containing Novozym or Flavourzyme (200 mg/100 g rice) before cooking. The contents of γ-oryzanol (ORY), phytic acid (PA), p-coumaric acid (pCA), ferulic acid (FA) and total phenolic compounds (TPC) were determined by HPLC-PDA (for ORY), enzymatic assays (PA) and HPLC-DAD-FLD-ECD (pCA, FA, and TPC). Phenolic compounds were quantified as soluble (free and conjugated) and insoluble-bound fractions to assess changes in their distribution following enzymatic treatment.

Overall, enzymatic soaking preserved the bioactive profile of cooked brown rice, with no significant differences in the levels of the identified compounds among treatments (Figure 1). Similarly, most phenolic fractions showed no significant changes, except for the soluble phenolic fraction in the free fraction ($p < 0.05$), with Flavourzyme-treated samples showing lower values (16.58 ± 0.18 mg GAE/100 g) than in Novozym-treated samples (26.57 ± 0.22 mg GAE/100 g). The increasing trend observed in free TPC (TPCf) suggests that enzymatic rice treatment may modify the distribution between free and conjugated phenolic compounds within the free soluble fraction. Nevertheless, this behaviour did not significantly affect the overall soluble TPC (Figure 1.B).

The results indicate that enzymatic soaking does not compromise the overall bioactive composition of cooked brown rice, although it may influence the partitioning of soluble phenolic compounds.

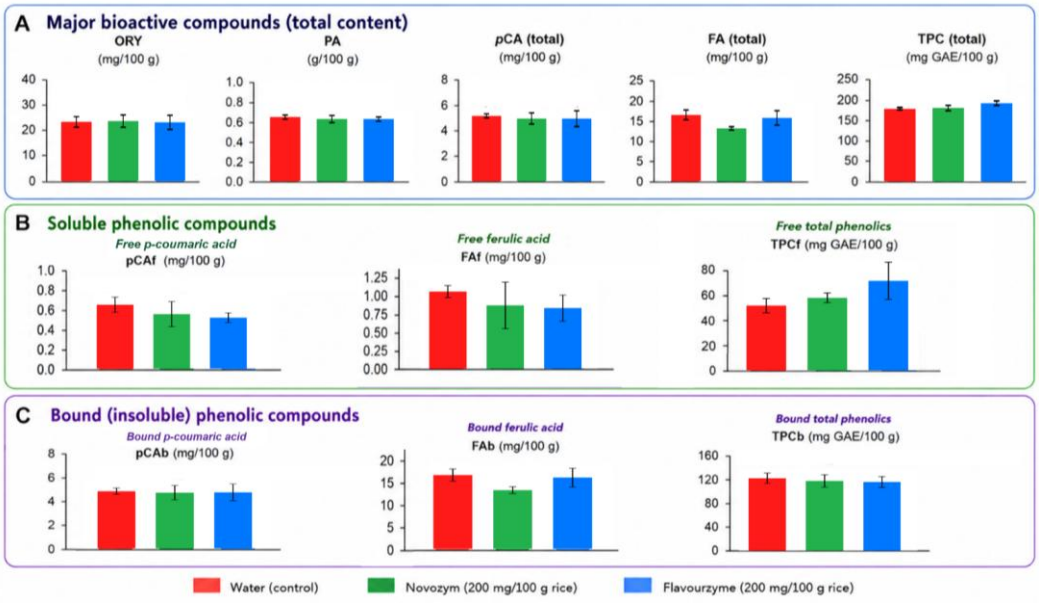


Figure 1 – Bioactive composition in treated brown rice sample. A - Major bioactive compounds (ORY: gamma oryzanol; PA: phytic acid; pCA: p-coumaric acid. FA: ferulic acid; TPC: total phenolic content expressed in gallic acid equivalents/100 g). B – Soluble phenolic compounds (f: free and conjugated). C -Insoluble phenolic compounds (b: bounded). Values are mean with standard deviation error bars (n=2). Different soaking solutions presented no significant differences.

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Tradition meets convenience: technological challenges behind a microwavable pastel de nata

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Pastel de nata is an emblematic product of Portuguese pastry, defined by the contrast between a crisp laminated puff pastry shell and a smooth, high moisture custard. Re-engineering this traditional product into a fully baked, frozen and microwave-regenerable format offers an opportunity for the technological valorisation of food heritage, but also a complex technological challenge due to the intrinsic instability of the custard–pastry system. During frozen storage and microwave regeneration, the high moisture gradient between both matrices, combined with preferential microwave heating of the aqueous custard phase and limited surface drying, promotes moisture migration, ice-related structural changes and pastry softening [1,2]. These events will compromise the characteristic crispness and flakiness of the pastry and promote custard instability, syneresis and structural collapse. To address these challenges, this work presents the technological strategy developed for a frozen, microwave-regenerable *pastel de nata*, based on the combined optimisation of functional ingredients[3] and processing conditions. The approach targeted three critical mechanisms: control of water mobility, reinforcement of the laminated pastry structure, and thermal stabilisation of the custard during microwave regeneration.

More than 94 formulation and process optimisation approaches were evaluated, including texturing proteins, dietary fibres, hydrocolloids, modified starches and different lipid systems. The most promising pastry system combined soy protein and an inulin-oat fibre blend for structural reinforcement and water management, together with xanthan gum to improve freeze–thaw stability and limit ice-crystal formation. Traditional margarine was replaced by anhydrous butter (>99.9% fat), thereby reducing the water introduced by the lipid phase and improving lamination, layer separation and overall pastry baking. Complete baking before freezing was identified as a critical processing step, since underbaked pastry could not develop crispy texture during microwave regeneration. Following frozen storage and microwave regeneration, the combined reformulation reduced pastry water activity (a_w) from 0.570 in the original formulation to 0.292.

In the custard, wheat flour was replaced by a freeze–thaw-stable phosphate-modified starch and combined with HPMC, improving water management and matrix stability and reducing a_w from 0.989 to 0.905. Among the hydrocolloids evaluated, HPMC showed the most promising behaviour for thermal stabilization of the custard, as its thermogelling properties helped reinforce the matrix during microwave heating and control heat-induced structural disruption.

Formulation optimization was complemented by reducing the mixing time and speed to limit excessive gluten development and dough elasticity, by introduction of resting periods between lamination steps, optimization of baking time–temperature conditions and adjustment of blast-freezing parameters. This integrated strategy preserved a crisp laminated pastry after microwave regeneration for up to two months of frozen storage, despite pastry Aw progressively increasing to values above 0.60. This increase highlights the persistence of moisture migration during storage and identifies it as a key limiting factor for longer-term stability. Hydrophobic barriers based on anhydrous butter are therefore currently being evaluated to delay moisture transfer across the custard–pastry interface and further extend product shelf-life.

Overall, bringing *pastel de nata* from tradition to microwave convenience is challenging due to the interaction of two fragile matrices and critical processing steps. Overcoming these technological constraints creates an opportunity to develop a more convenient and potentially energy-efficient product while preserving its traditional sensory identity.

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Rheological and Functional Behaviour of Legume Flours: Impact of Processing and Structural Organisation

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The growing demand for sustainable plant-based ingredients has increased interest in legume flours with technological properties tailored to specific food applications. This study investigated the effects of botanical origin and processing history on the functional and rheological behaviour of grass pea (GP), chickpea (CP1, CP2 and CP3), and lupin (L) flours. GP and CP1 were supplied directly as flours, whereas CP2 was obtained by milling dry chickpea grains through a 1 mm sieve. CP3 was produced by soaking, drying and milling chickpea grains, while L was obtained by soaking, cooking, drying and milling lupin grains. All raw materials were supplied by the same producer. Before analysis, all flour samples were standardised by sieving through a 250 µm mesh.

Moisture content, water, oil and milk absorption capacities, swelling power, foaming and emulsifying properties, and gelation behaviour were determined. Functional analyses were conducted using three independently prepared replicates, except for gelation, which was evaluated in duplicate. For rheological analysis, the flours were dispersed in water at a concentration of 6 g/100 mL. Three independent dispersions were prepared for each flour and measured in duplicate. Oscillatory stress sweeps were first performed to determine the linear viscoelastic region, followed by frequency sweeps from 0.01 to 100 Hz at 20 ± 1 °C using serrated parallel plates with a diameter of 20 mm.[1] Data were analysed by one-way analysis of variance, followed by Tukey's multiple-comparison test, with statistical significance established at $p < 0.05$.

The flours exhibited distinct functional profiles, reflecting the combined effects of botanical origin and processing. L flour showed the highest water absorption capacity (2.5 g/g), milk absorption capacity (4.9 g/g) and swelling power (4.2 g/g), demonstrating a strong affinity for aqueous phases. This response may reflect the composition of lupin flour and structural modifications induced by soaking and cooking, which can increase the accessibility of hydrophilic groups[2]. Among the chickpea flours, CP3 showed greater water absorption (2.1 g/g), milk absorption (2.2 g/g) and swelling power (3.2 g/g) than CP1 and CP2. Thus, soaking followed by drying and milling may have disrupted the flour matrix and increased the availability of water-binding sites. CP3 also presented the lowest phase separation (25.9 %), suggesting a lower tendency towards emulsion destabilisation, although phase separation, creaming and sedimentation should be interpreted jointly[2].

GP displayed the highest foaming capacity (28.8%) while maintaining high foam stability (94.5 %), supporting its potential in aerated formulations. Oil absorption varied only slightly among samples (1.00–1.10 g/g), indicating that this property was less responsive to flour type and processing than hydration-related properties. Gelation behaviour also differed, with CP2 presenting the lowest recorded values at all concentrations tested. Because this assay and oscillatory rheology evaluate different aspects of structure formation, their rankings should not be interpreted as directly equivalent.

All dispersions showed predominantly solid-like behaviour, with the storage modulus (G') remaining above the loss modulus (G") throughout the frequency range. Both moduli increased with frequency, indicating weak gel-like, frequency-dependent systems[1]. At 1 Hz, G' values were 13.4, 38.8, 28.3, 29.5 and 25.9 kPa for CP1, CP2, CP3, GP and L, respectively, while the corresponding G" values were 1.69, 4.22, 2.37, 3.06 and 2.85 kPa. CP2 therefore formed the strongest viscoelastic network, CP1 the weakest, and CP3, GP and L showed intermediate responses. The higher hydration capacity of CP3 did not result in the highest elastic modulus, confirming that water absorption and network strength are not directly proportional. Soaking may enhance hydration while also promoting the solubilisation or loss of components involved in network formation. Differences among samples are likely related to starch, protein and fibre composition, as well as processing-induced changes in molecular interactions and particle structure.

Overall, botanical origin and processing generated distinct hydration, interfacial and viscoelastic profiles. L appears promising for products requiring high water binding, GP for aerated systems, CP3 for formulations requiring hydration and reduced phase separation, and CP2 for structured products requiring greater elastic strength. CP1 may be more appropriate

for less-structured systems. These findings provide a basis for selecting legume flours according to technological function, although performance should be validated in formulated food matrices.

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Effect of dried fig and aromatic plants on the volatile profile of cured sausages made from Churra Algarvia sheep meat

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The use of ingredients associated with Algarve gastronomy can contribute to enhancing the value of meat products derived from native breeds [1]. Among these ingredients, dried figs [2] and aromatic plants can significantly alter the volatile fraction and, consequently, the aromatic identity of the cured sausages. This study aimed to evaluate the effect of adding dried figs and using thyme or fennel on the volatile compound profile of dry-cured sausages made from Churra Algarvia sheep meat.[3]

Four formulations were studied: thyme, thyme with dried fig, fennel, and fennel with dried fig. The volatile profile was determined in 36 samples, nine per formulation, considering 127 compounds. The data were subjected to principal component analysis based on the correlation matrix, retaining three components and applying Varimax rotation. The scores for the three components were compared across formulations using the Kruskal–Wallis test, followed by Dunn's procedure with Bonferroni correction.

The first three components explained 54.25% of the total variability, with the first component accounting for 23.62%, the second for 16.27%, and the third for 14.37%. The first component was primarily associated with phenolic compounds, including 2-methoxy-5-methylphenol, phenol, 2,6-dimethoxyphenol, 4-ethyl-2-methoxyphenol, and dimethylated phenols, suggesting a dimension related to smoking and thermal processing compounds, that was applied in all the formulations, thus the lack of differences. The second component was mainly associated with alcohols and ethyl esters formed during fermentation and ripening, reflecting microbial carbohydrate and amino-acid metabolism and subsequent esterification reactions. Although this component accounted for 16.27% of the total variance, its scores did not differ significantly among formulations, suggesting that the variation in fermentation and ripening-related compounds was not systematically associated with the ingredients tested. The third component primarily distinguished the type of aromatic plant used. Positive scores were associated with thyme-containing formulations and were characterized by thymol, together with 2-propenal, hexanal, 2-methylbutanal, and 3-methylbutanal. Negative scores were associated with fennel-containing formulations and were characterized by estragole, anethole, eucalyptol, and other fennel-related terpenes. The addition of dried fig appeared to modulate this pattern, particularly within the thyme formulations, but the dominant contrast captured by the third component was between thyme and fennel-based products.

No significant differences were observed among formulations in the scores of the first component ($p = .576$) or the second component ($p = .098$). In contrast, F3 scores differed significantly among formulations ($p < .0001$; Figure 1), increasing from the fennel formulation, which showed the most negative values, to the thyme-and-dried fig formulation, which showed the most positive values. Pairwise comparisons indicated that the fennel formulation differed significantly from both thyme formulations, while fennel-and-dried fig formulation differed from thyme-and-dried fig formulation. No significant differences were observed between formulations with and without dried fig within the same aromatic plant.

This pattern indicates that the type of aromatic plant was the main factor differentiating the volatile profiles. Negative F3 scores were associated with fennel-related compounds, particularly estragole, anethole, and eucalyptol, whereas positive scores were associated with thyme-related compounds, especially thymol, together with several aldehydes. Dried fig appeared to modulate this contrast by shifting the scores towards more positive values, particularly in the thyme formulation, although its effect was not statistically significant within either aromatic-plant group. Overall, the multivariate approach condensed the information provided by 127 volatile compounds and identified the principal chemical patterns underlying the differentiation among formulations.

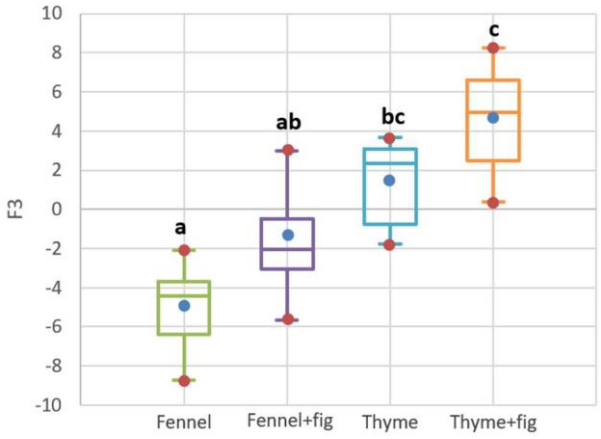


Figure 1 - Distribution of the scores of the third principal component (F3) according to sausage formulation. Boxes represent the interquartile range, horizontal lines indicate the median, whiskers represent the observed range, and blue dots indicate the mean. Different lowercase letters indicate significant differences between formulations according to Dunn's multiple-comparison procedure with Bonferroni correction following a significant Kruskal–Wallis test ($p < .05$).

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Fatty acid composition and nutritional lipid indices of dry-cured sausages made with Churra Algarvia sheep meat

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The nutritional evaluation of food lipids should not rely exclusively on their total saturated, monounsaturated, or polyunsaturated fatty acid contents, since individual fatty acids differ in their potential physiological effects.[1] Conventional ratio, such as PUFA/SFA and n-6/n-3, together with the atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H), provide an integrated characterization of lipid quality. Lower AI and TI values and higher h/H values are generally regarded as indicators of a more favorable fatty acid balance, although these indices should be interpreted as nutritional screening tools rather than direct predictors of health outcomes.[2]

This study aimed to characterize the fatty acid composition and nutritional lipid indices of dry-cured sausages produced with Churra Algarvia sheep meat and Alentejano pig fat.

Four formulations were produced: thyme, thyme with dried fig, fennel, and fennel with dried fig. The base formulation contained 75% Churra Algarvia sheep meat and 25% Alentejano pig fat. The sausages were smoked and dry-cured under controlled conditions. One composite sample from each formulation was analyzed. Total lipids were extracted and converted into fatty acid methyl esters, which were analyzed by gas chromatography with flame-ionization detection. Individual fatty acids were identified by comparison with reference standards and quantified as g/100 g of product. The MUFA/SFA, PUFA/SFA, and n-6/n-3 ratios, together with AI, TI, and h/H, were subsequently calculated. Owing to the composite sampling design, the results were interpreted descriptively and expressed as the ranges observed across formulations.

The sum of the main fatty acid classes ranged from approximately 20 to 34 g/100 g of product. Saturated fatty acids predominated, ranging from 15 to 29 g/100 g, followed by monounsaturated fatty acids, from 4.5 to 10.0 g/100 g, and polyunsaturated fatty acids, from 0.25 to 1.00 g/100 g. Trans fatty acids were below 0.01 g/100 g in all samples. Palmitic, stearic, and oleic acids were the predominant individual fatty acids. Omega-3 fatty acids ranged from 0.06 to 0.61 g/100 g, whereas omega-6 fatty acids ranged from 0.15 to 0.41 g/100 g. The MUFA/SFA ratio varied from 0.16 to 0.67, the PUFA/SFA ratio from 0.01 to 0.04, and the n-6/n-3 ratio from 0.67 to 4.17. AI and TI ranged from 1.41 to 4.18 and from 2.38 to 9.84, respectively, while h/H ranged from 0.25 to 0.97.

The products were characterized by a predominance of saturated fatty acids, a relevant monounsaturated fraction, low polyunsaturated fatty acid contents, and negligible trans-fat. The combined use of conventional fatty acid ratios and nutritional lipid indices provided a more informative description of the lipid fraction than fatty acid class totals alone. Nevertheless, the observed ranges should be interpreted as descriptive compositional variability and not as demonstrated effects of dried fig or aromatic herbs. These results provide baseline information for the nutritional characterization and future optimization of drycured products made with Churra Algarvia sheep meat.[3]

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Effect of Solvent Extraction and Plant Fraction on Total Phenolic Content and Antioxidant Activity of Coriander (*Coriandrum sativum L.*) and Parsley (*Petroselinum crispum (Mill.) Nym.*) from the Portuguese Saloia Region

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Aromatic herbs are valuable sources of bioactive compounds and may contribute significantly to natural antioxidant intake. Among them, coriander (*Coriandrum sativum* L.) and parsley (*Petroselinum crispum* (Mill.) Nym.) are widely used culinary herbs with recognised functional value. The presence of phenolic compounds, flavonoids, and other bioactive metabolites contributes to their antioxidant capacity. Several other biological activities, including anti-inflammatory, hypoglycemic, hypolipidemic, neuroprotective, and anticancer effects, have also been reported.[1,2] These attributes highlight their potential value as functional foods and medicinal aromatic plants.

This study investigated the effects of solvent extraction (SE; aqueous and methanolic) and plant fraction (PF; leaves and stems) on the total phenolic content (TPC; mg GAE/100 g) and antioxidant activity of coriander and parsley cultivated in the Saloia Region of Sintra, Portugal. Antioxidant capacity was determined using DPPH, FRAP, and ABTS assays, with results expressed as µmol/100 g, mmol $FeSO_4 \cdot H_2O$/100 g, and µmol TE/100 g fresh weight, respectively.[3] Phenolic and organic acid profiles are currently under analysis, providing further insight into the compounds underlying the antioxidant activity of coriander and parsley extracts. The statistical analysis was performed using STATISTICA software version 12 (StatSoft Inc., Tulsa, OK, USA). A two-way analysis of variance (ANOVA) was conducted with SE and PF as fixed factors, including their interaction (SE×PF). When significant effects were detected by ANOVA ($p<0.05$), post-hoc comparisons were performed using Tukey's test.

In coriander, TPC, FRAP, and DPPH were significantly affected by the SE×PF interaction ($p<0.05$). For TPC, leaves showed the highest values in both water and methanolic extracts, with no significant differences between extract types, averaging 72.8 mg GAE/100 g. In contrast, stems showed significantly higher TPC in methanolic extracts than in water extracts, with values of 45.8 and 30.9 mg GAE/100 g, respectively. A similar trend was observed for FRAP, with leaves generally showing greater reducing capacity than stems, and the highest value recorded in methanolic leaf extracts (17.8 mmol $FeSO_4 \cdot H_2O$/100 g). In the DPPH assay, methanolic extracts from both leaves and stems showed the highest antioxidant activity, with no significant differences between plant parts, averaging 3394 µmol TE/100 g fresh weight. Conversely, water stem extracts showed the lowest DPPH activity. ABTS activity was significantly affected only by SE ($p<0.05$), with no significant effect of PF or the SE×PF interaction ($p>0.05$). Overall, methanolic extracts showed higher ABTS activity than water extracts, averaging 3244 µmol TE/100 g fresh weight.

For parsley, TPC was significantly affected only by PF ($p<0.001$), with no significant effect of SE or the SE×PF interaction ($p>0.05$). Leaves showed significantly higher TPC than stems in both water and methanolic extracts, averaging 57.5 mg GAE/100 g, whereas stems averaged 20.3 mg GAE/100 g. FRAP, DPPH, and ABTS activities were significantly affected by the SE×PF interaction ($p<0.05$). For FRAP, methanolic leaf extracts showed the highest reducing capacity (16.76 mmol $FeSO_4 \cdot H_2O$/100 g), while water stem extracts showed the lowest value (2.32 mmol $FeSO_4 \cdot H_2O$/100 g). A similar pattern was observed for DPPH, with methanolic leaf extracts presenting the highest antioxidant activity (3475 µmol TE/100 g fresh weight). For ABTS, water and methanolic leaf extracts, as well as methanolic stem extracts, showed similarly high antioxidant activity, averaging 3199 µmol TE/100 g fresh weight.

Overall, these findings suggest that leaves are the main reservoirs of phenolic compounds and antioxidant activity in both coriander and parsley. However, the extraction solvent strongly influenced the antioxidant response, particularly in FRAP, DPPH, and ABTS assays. The higher activity observed in methanolic extracts indicates that methanol was more effective in extracting antioxidant compounds, especially those involved in radical scavenging and reducing mechanisms. The differences among assays further suggest that antioxidant activity is not determined solely by total phenolic content, but also by the chemical nature of the extracted compounds and their specific reaction mechanisms.

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Phenolic composition and antioxidant activity of functional flours from Brassica surpluses (Galega and Penca)

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Brassica surpluses are abundant, perishable and rich in bioactive compounds, making them attractive raw materials for functional flours within a circular-economy framework [1]. Galega kale (*Brassica oleracea* var. acephala) and Penca cabbage (*Brassica oleracea* var. *costata*), characteristic of the Southern European Atlantic Diet, were valorised into dried flours to assess how cultivar, drying temperature and blanching modulate their phenolic composition and lipid peroxidation inhibitory activity, and which factor has the greatest impact. Fresh leaves of each cultivar were convective hot-air dried at 60, 70 or 80 °C, with and without blanching (95°C, 2 min, 1:7 leaf to water ratio) until a final moisture content below 4% was reached. Phenolic compounds were extracted through hydroethanolic maceration (ethanol:water 80:20, *v/v,* 1 hour). After solvent evaporation and lyophilization, the obtained extract powder was analysed by HPLC-DAD-ESI-MS^n. Hydroxycinnamic acid derivatives and flavonols (summed by class) were determined and expressed as mg/g of extract. The lipid peroxidation inhibitory activity was evaluated by the thiobarbituric acid reactive substances (TBARS) assay and expressed as EC_{50} value (mg/g). Results (mean ± SD) were compared by one-way ANOVA with Tukey's HSD (p<0.05), and the contribution of each factor was quantified by three-way factorial ANOVA (partial η^2).

Cultivar was the primary driver for the phenolic concentration (Fig. 1a). *Galega* was far richer in total phenolics (29.9–39.3 mg/g of extract) than *Penca* (6.7–15.2 mg/g of extract) and contained abundant kaempferol and quercetin derivatives, whereas Penca was almost devoid of quercetin glycosides; these cultivar-specific profiles match previous reports for the two botanical varieties[2].

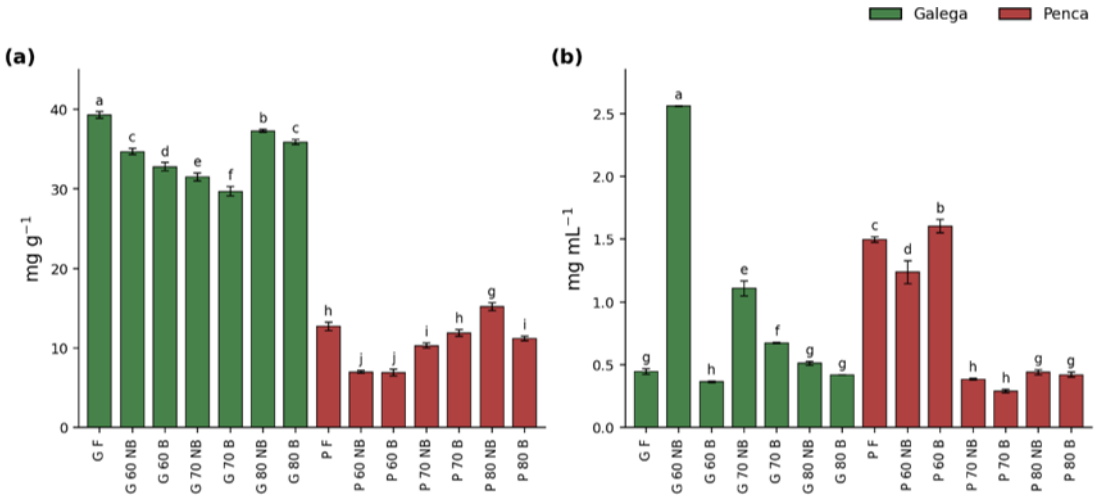


Figure 1 - Total phenolic content (a) and TBARS EC_{50} (lower values = higher activity) (b) of *B. oleracea* flours. Mean ± SD; different letters indicate significant differences (Tukey's HSD, p < 0.05). G, Galega; P, Penca; F, fresh; 60/70/80, drying temperature (°C); B/NB, blanched/not blanched.

The factorial analysis attributed up to 95% of the variance in total phenolics to cultivar. In contrast, TBARS (Fig. 1b) was influenced mainly by drying temperature (partial η^2 = 44%): the strongest activity (lowest EC_{50}) occurred at 70–80 °C and in blanched flours. This result could be associated with the inactivation of polyphenol-related oxidative enzymes and the formation of thermally derived compounds with antioxidant activity during drying[3]. Phenolic contents and TBARS were not directly correlated, suggesting that specific or newly formed compounds, or synergistic interactions among them, drive the antioxidant response rather than the concentration of individual bioactive compounds.

Cultivar choice governs the phenolic richness of the flours, with Galega being the preferred raw material, whereas drying temperature and blanching act as a secondary lever for tuning antioxidant activity. Convective drying at 60–80 °C proved a feasible route to valorise these *Brassica* surpluses into phenolic-rich functional flours.

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Methodological optimization of bromelain extraction and purification from Azorean pineapple stem for biotechnological and health applications

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Pineapple (*Ananas comosus* L.) is a tropical perennial plant belonging to the Bromeliaceae family.[1] This plant is an excellent source of several biologically active and valuable compounds, one of which is bromelain, a group of proteolytic enzymes that have been extensively studied across various scientific research centers. Bromelain exhibits a range of biotechnological, pharmacological and clinical properties, including inhibition of malignant cell growth, anti-inflammatory action, fibrinolytic activity, skin debridement, antioxidant and digestive effects.[2] Additionally, bromelain has been reported to inhibit the entry of SARS-CoV-2 virus into host cells by suppressing TMPRSS2 proteases present on the host cell surface. This inhibition may also prevent the cleavage of viral glycoproteins into viral functional proteins, necessary to viral replication, thereby reducing the ability of the virus to infect cells.[3]

The aim of this study was to compare the bromelain extraction/purification yields using cationic exchange chromatography with two different methodologies (with and without cold ethanol precipitation) and, also to investigate the different physiological stages of the plant that produces more bromelain content. The extraction was performed using previously dried pineapple stem material, followed by grinding and the addition of a 10% sodium chloride solution, freezing at −20 °C for 24 hours, and subsequently centrifuged to obtain a bromelain-rich supernatant. The purification process included dialysis steps using a membrane with a molecular weight cut-off of 12–14 kDa, followed by either ethanol precipitation (method 1) or without ethanol precipitation (method 2) and subsequently purification by cation-exchange chromatography.

Table 1 shows the results of the two methods revealing that the most effective (higher yield) of the extraction and purification of bromelain was cationic-exchanged chromatography without ethanol precipitation (method 2), in which it was possible to extract approximately the double of bromelain (6.67% and 14.69% as compared to 3.61% and 7.01% for method 2 and method 1, respectively). The sample that completed the vegetative cycle and the stem that was maintained in the soil presented similar values in both methods.

Table 1 – Bromelain content using two different extration and purification methodologies A, plant that produces fruit; B, plant that does not produce fruit; C, plant completed the vegetative cycle and the stem was maintained in the soil; CEC, Cation Exchanged Chromatography.

Sample	CEC extraction and purification	
	Ethanol cold precipitation	Without ethanol precipitation
A	3.61%	6.67%
B	7.01 %	14.69%
C	3.04%	2.96%

In conclusion, the sample obtained from the non-fruit-producing plant showed the highest bromelain content, as the enzyme is concentrated only in the stem, whereas the sample from the plant that produces fruit, bromelain is distributed between stem and fruit. The sample from the plant that completed its life cycle and the stem remaining in the soil exhibited the lowest bromelain content because the plant stem is depleted.

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Enhancing theaflavin content in Camellia sinensis leaves through rehydration and secondary withering: effects on composition and antioxidant activity

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Theaflavins, key components of *Camellia sinensis,* are polyphenolic compounds found abundantly in black teas, with several reported biological activities. Theaflavins have been shown to protect against oxidative stress-related diseases such as cancer, cardiovascular diseases and neurodegenerative disorders, and ultimately the reduction of coronavirus activity.[1] It is well known that *Camellia sinensis* black tea is one of the most widely consumed beverages in the world and has been a popular beverage for millennia. The production of black tea is mainly divided into three steps: plucking the fresh leaves, withering, and enzymatic oxidation, commonly referred to as fermentation. Withering and fermentation are essential steps in tea production, significantly shaping the development of its unique flavour profile.[2] Catechin content decreases during the withering process, while theaflavins and their polymeric forms, thearubigins, are formed. These two tea components are crucial for the colour, brightness, briskness, and strength characteristics of black tea.[3]

This study evaluated the effect of leaf rehydration and second withering time on the theaflavin content and antioxidant activity of Azorean black tea, and how rehydrating tea leaves after withering can be optimised to enhance levels of theaflavin-3,3'-O-digallate (TF-3,3'-DG), and total theaflavins (TFs) given their potential to increase the body's defense against viruses.[2] All samples were withered at room temperature followed by an oxidation step for 3 hours (h) and the rehydration was performed within 15 seconds by water spray. Regarding antioxidant properties based on free radical scavenging activity (DPPH-FRSA), the highest antioxidant capacity was observed in the non-rehydrated sample (EC_{50} = 3.72 ± 0.06 µg/mL), followed by the sample rehydrated after an 8 h withering and a subsequent 6 h second withering (EC_{50} = 5.73 ± 0.28 µg/mL). In comparison, the treatment involving a 12 h withering, rehydration, and a 3 h second withering exhibited slightly lower antioxidant activity (EC_{50} = 5.76 ± 0.01 µg/mL). A similar trend was observed for ferric reducing antioxidant power (FRAP), where the non-rehydrated sample showed the highest activity (EC_{50} = 6.80 ± 0.03 µg/mL), followed by the 12 h withering + rehydration + 3 h second withering treatment (EC_{50} = 9.87 ± 0.01 µg/mL), and the 8 h withering + rehydration + 6 h second withering sample (EC_{50} = 10.36 ± 0.26 µg/mL). For ferrous ion-chelating activity (FIC), results were comparable between the 12 h withering + rehydration + 3 h second withering sample (EC_{50} = 0.41 ± 0.01 mg/mL) and the 8 h withering + rehydration + 6 h second withering sample (EC_{50} = 0.38 ± 0.01 mg/mL), while the non-rehydrated sample yielded an EC_{50} of 0.50 ± 0.02 mg/mL. For theaflavin quantification, high-performance liquid chromatography was used and the results revealed that the non-rehydrated sample presented the lowest TF-3,3'-DG and total TFs (6.31 ± 0.47 and 22.06 ± 0.91 mg/g dry weight (DW), respectively), followed by 12 h withering, rehydration and a 3 h second withering (9.82 ± 0.27 and 28.34 ± 1.88 mg/g DW, respectively). Similarly, the 8 h withering + rehydration + 6 h second withering cycle increased TF-3,3'-DG to 11.67 ± 0.38 mg/g DW and presented a slightly lower total TFs (25.80 ± 1.67 mg/g DW) than the 12 h + 3 h treatment. Therefore, the rehydration process positively impacted both TF-3,3'-DG and total theaflavin contents. This study highlights the potential for developing a novel Azorean tea by optimising processing conditions to maximize TF-3,3'-DG content, a compound reported by several Research Centers to inhibit key components of viral replications.

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Biochemical composition and antioxidant activity of Azorean Lepidium sativum (L.) seeds as a functional food supplement

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Osteoarthritis is widespread globally and is characterized by the progressive catabolic breakdown of cartilage proteoglycans, and standard treatments remain predominantly palliative and may unpredictably contribute to further cartilage degeneration. *Lepidium sativum* (LS), commonly known as "garden cress", belongs to the Brassicaceae family (Cruciferae), and is being recognized as a functional food due to its high nutritional value, driven by the presence of essential macro- and micronutrients.[1]

According to World Health Organization, in 2019, about 530 million of people worldwide were living with osteoarthritis (OA), the most advaced stage of arthritis, and approximately 345 million is experiencing moderate to severe levels, that is marked by the depletion of proteoglycan (PG) molecules and their glycosaminoglycan (GAG) components from cartilage.[2,3]

As far as the authors are aware, this is the first study on Azorean *Lepidium sativum*, and the aim of this study was to investigate the biochemical composition of LS seeds, in order to better understand its high nutritional value as a food supplement and the basis of their potential anti-rheumatic, considering that LS seeds have been used in the Azores Islands for decades as an anti-rheumatic remedy. The samples were particularly rich in α-linolenic acid (38.40%) and eicosenoic acid (13.40%), with an ω3/ω6 ratio of 2.87 that act as mediators of the autoimune disorders. The predominant phytosterols, structurally similar to human cholesterol, were β-sitosterol, campesterol, and stigmasterol with concentrations ranging from 42.50 to 2.15% and traces (<0.1%) of brassicasterol, that compete with dietary cholesterol lowering the risk of aterosclerose. Vitamin analysis (mg per 100 g of dry weight) revealed the contents: vitamin B1 (0.73), B2 (0.40), B6 (1.04), B3 (12.23), B9 (0.12), and α-tocopherol (8.41) that are significant in relieving the pain and stiffeness of the patients.

The **Table 1** shows the values of antioxidants, total phenolic and total flavonoid contents (TPC and TFC, respectively) of LS seeds. The seeds exhibited moderate antioxidant activity, with EC_{50} values of 50.77 and 64.00 µg/mL for free radical scavenging activity and ferric reducing antioxidant power assays, respectively. TPC and TFC were 63.93 mg of gallic acid equivalents (GAE) and 12.00 mg of rutin equvalents (RE) per g of dry extract (DE). Additionally, the presence of essential B-vitamins, α-tocopherol (vitamin E), and moderate antioxidant activity, driven by total phenolic and flavonoid contents, suggests a synergistic therapeutic effect. Overall, the phytonutraceutical profile of LS suggests potential antioxidant, and therapeutical effects which may reduce the pain, supporting their traditional use in the management of rheumatic disorders. However, individuals may respond differently to this treatment, possibly as a result of lifestyle choices, risk factors, or the degree of established joint damage. Nevertheless, its diverse bioactivities make it a promising candidate for the development of nutraceuticals and functional foods.

Table 1 – Antioxidant activities (FSRA - free radical scavenging activity, FRAP – ferric reducing antioxidant power), TPC - total phenolic content, TPC - total flavonoid content) in *Lepidium sativum* seed extracts.[a]

	Lepidium sativum	Ascorbic Acid
FRSA (EC_{50}, µg/mL)	50.77 ± 0.26	2.11 ± 0.04
FRAP (EC_{50}, µg/mL)	64.00 ± 1.10	3.91 ± 0.57
TPC (mg GAE/g DE)	63.93 ± 0.42	-
TFC (mg RE/g DE)	12.00 ± 0.28	-

[a]Values are mean ± SD (*n=4*); EC_{50}, half-maximal effective concentration; GAE, gallic acid equivalents; RE, rutin equivalents; DE, dry extract.

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Valorization of Traditional Portuguese Resources Through Bread Formulations Enriched with Acorn Flour and Essential Oils

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There is a growing interest in valorising traditional Portuguese food resources as ingredients for the development of more functional and nutritious foods. Acorn flour, historically consumed in several Mediterranean regions, represents an underutilized raw material with significant potential for valorisation due to its abundance and nutritional value[1]. Likewise, Portuguese aromatic and medicinal plants constitute a valuable source of essential oils (EOs), which are widely recognised for their antioxidant properties and other bioactive activities. However, owing to their volatility and susceptibility to degradation, the incorporation of EOs into food systems requires encapsulation[2]. Therefore, the aim of this work was to develop a bread formulation combining the traditional use of acorn flour with encapsulated EOs obtained from Portuguese flora (Figure 1).

EOs were chemically characterised by gas chromatography-mass spectrometry (GC-MS), and their antioxidant activity was evaluated using spectrophotometric methods. Two of the analysed EOs exhibited high antioxidant activity, reinforcing their suitability as natural ingredients for food applications. Microencapsulation was performed using bacterial cellulose (BC) and methylcellulose (MC), and the stability of the encapsulated systems was assessed by visual observation and diffusion-wave spectroscopy. Bread formulations were then prepared using wheat flour enriched with encapsulated EOs, and others using also acorn flour, allowing to evaluate the compatibility of the encapsulated systems with different flour matrices. Breads containing encapsulated EOs retained considerable antioxidant capacity after baking, indicating that the encapsulation strategy effectively protected the bioactive compounds against thermal degradation.

Overall, this work demonstrates how traditional Portuguese food resources can be successfully combined with modern encapsulation technologies to develop innovative products with enhanced antioxidant potential, contributing to both food innovation and to the valorisation of Portuguese food heritage.

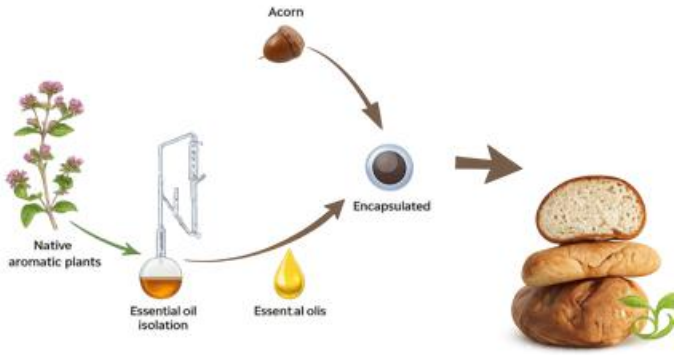


Figure 1 - Schematic picture of the work.

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Optimization of Wheat Bread Formulations Enriched with Acorn and Lupin Flours Using a Mixture Design

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The growing demand for healthier and more sustainable food products has stimulated the development of bakery products enriched with alternative plant-based ingredients.[1] In this context, acorn and lupin flours have been investigated as partial substitutes for wheat flour in bread.[2,3] Acorn flour is rich in dietary fiber, unsaturated fatty acids, and antioxidant compounds, whereas lupin flour is characterized by its high protein and dietary fiber contents. Moreover, both raw materials are locally available and can contribute to the sustainability and diversification of agri-food systems.

The main objective of this work was to develop nutritionally improved wheat breads enriched with acorn and lupin flours, aiming to increase their protein, dietary fiber, and bioactive compound contents while maintaining acceptable processing performance and technological quality.

The effect of acorn and lupin flour incorporation on bread quality was evaluated, and the optimal flour blend was identified using an I-optimal mixture design. Bread formulations containing different proportions of wheat, acorn, and lupin flours were prepared. Based on literature data and preliminary experiments, the combined incorporation of acorn and lupin flours was limited to a maximum of 30% of the total flour mixture.

The resulting breads were characterized in terms of specific volume, texture profile, moisture content, crust and crumb color, and crumb structure. In parallel, the viscoelastic properties of the doughs were evaluated by oscillatory rheology. Polynomial mixture models were fitted to the experimental data to describe the relationships between flours and the measured responses, allowing the identification of significant mixture effects, interactions among flour components, and optimal formulation regions.

Overall, the incorporation of acorn and lupin reduced the specific volume and adversely affected the textural properties of the breads, with acorn flour showing a more pronounced negative effect than lupin flour. Nevertheless, the mixture design demonstrated that breads with acceptable technological quality could be obtained using formulations containing approximately 10% lupin flour and 2.5% acorn flour. Sensory evaluation of the optimized formulation will be carried out in future work to assess its consumer acceptability.

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Effect of carrot powder incorporation on the chemical and sensory properties of gluten-containing and gluten-free pasta

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The development of foods with improved nutritional quality has become an important research area in food science, driven by increasing consumer interest in functional and health-promoting products. Pasta is one of the most widely consumed staple foods worldwide and represents an interesting matrix for nutritional improvement due to its regular consumption and versatility. However, conventional pasta is mainly produced from wheat flour, limiting its suitability for individuals with celiac disease or gluten intolerance. Consequently, the development of gluten-free alternatives and the incorporation of natural ingredients with bioactive compounds have gained increasing attention. Vegetable-based ingredients are increasingly explored as sources of antioxidants, dietary fibre, vitamins, minerals, and other beneficial compounds. Carrot is particularly promising due to its high content of carotenoids, especially β-carotene, as well as phenolic compounds with antioxidant activity[1]. These compounds are responsible for its characteristic orange colour and functional potential. Buckwheat is also recognised as a valuable gluten-free ingredient due to its nutritional composition and presence of bioactive compounds, including flavonoids and phenolics[2]. Therefore, combining buckwheat flour and carrot powder may improve the nutritional profile of pasta while providing a gluten-free option.

The aim of this study was to evaluate the influence of carrot powder addition on the chemical composition and sensory characteristics of pasta produced with wheat and buckwheat flour. Six formulations were developed with carrot powder at different levels (0%, 5%, and 10%) in both wheat-based and gluten-free formulations[3]. After processing, drying, and cooking, samples were analysed for nutritional composition, carotenoid content, phenolic compounds, and sensory acceptance.

Results showed differences between formulations. Gluten-free pasta exhibited a more favourable nutritional profile, mainly due to buckwheat flour, which is naturally richer in bioactive compounds than wheat flour[3]. The addition of carrot powder further increased carotenoid and phenolic contents[3], confirming its role as a functional ingredient. The enrichment effect observed with increasing carrot concentration is attributed to its natural content of carotenoids and phenolic compounds, associated with antioxidant activity. Similar findings have been reported in studies involving vegetable-enriched pasta, where functional ingredients improve nutritional value while maintaining product quality. Sensory evaluation considered appearance, colour, aroma, taste, texture, and overall acceptability. Wheat-based pasta showed higher overall acceptance than gluten-free samples, likely due to the structural role of gluten in texture formation and mouthfeel (**Figure 1**).

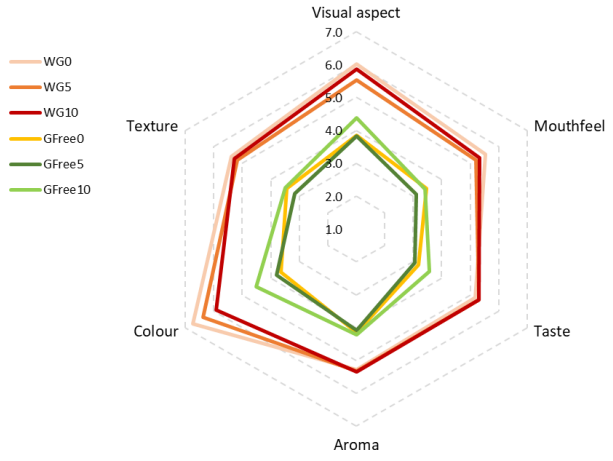


Figure 1 – Sensory descriptors for the six pasta samples. (Scale from 1 = extremely unacceptable to 9 = extremely acceptable).

However, carrot powder had a positive effect on sensory acceptance. Formulations with higher levels of carrot showed improved scores, suggesting that its addition enhanced both nutritional value and sensory appeal. This may be related to its colour and sensory properties, which increase product attractiveness. Similar trends have been reported in other vegetable-enriched pasta products, where formulation balance is essential between nutrition and consumer acceptance. Overall, results highlight the importance of balancing nutritional enhancement with sensory quality in pasta development.

Although gluten-free products are essential for specific dietary needs, maintaining desirable sensory characteristics remains challenging. The incorporation of natural ingredients such as carrot powder may help address this limitation.

In conclusion, carrot powder represents a promising ingredient for nutritionally enriched pasta. Its combination with wheat or buckwheat flour increased carotenoid and phenolic content while maintaining acceptable sensory properties, supporting its use in functional pasta development.

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Influence of Hydroethanolic Solvent Composition on the Extraction of Phenolic Compounds from *Crataegus monogyna* Berries

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Crataegus monogyna berries are recognized as an important source of phenolic compounds, including phenolic acids, flavonoids and other bioactive metabolites associated with antioxidant, cardioprotective and anti-inflammatory properties [1]. Due to the increasing interest in natural ingredients to be used for functional foods and nutraceutical applications, and the intrinsic potential of this berry, the development of efficient extraction strategies is essential to maximize the recovery of these compounds while preserving their phytochemical profile. Among the factors affecting extraction efficiency, solvent polarity plays a decisive role, with hydroethanolic mixtures generally providing higher extraction yields than pure solvents [2,3]. However, the influence of ethanol concentration on the extraction efficiency and phenolic profile of *C. monogyna* berries has not yet been fully elucidated. Thus, the present study aimed to evaluate the effect of different ethanol concentrations (0, 25, 50, 75 and 100%, v/v) on the extraction of phenolic compounds from *C. monogyna* berries. Fruits were frozen immediately after harvesting, freeze-dried and milled into a fine powder. The extraction was carried out by preparing 1% (w/v) suspensions of berry powder in the different solvents followed by homogenization using an Ultra-Turrax and the extraction was left to occur, at room temperature, in a tilt and roll shaker. After 30 min, the extracts were centrifuged and evaluated for total phenolic content (TPC), o-diphenolic compounds (o-DP) and total flavonoids (TF) by spectrophotometric assays. In addition, chromatographic fingerprints were obtained by HPLC-DAD at 280 and 320 nm to assess possible profile differences among extraction solvents. All analyses were performed in triplicate, and statistical significance was considered at $p < 0.05$. Ethanol concentration significantly influenced the extraction of TPC and o-DP. Water extracts exhibited the lowest contents, whereas all ethanol-containing solvents significantly improved the recovery of these compounds. No statistically significant differences were observed among extracts obtained with 25, 50, 75 and 100% ethanol, indicating that the presence of ethanol, rather than its absolute concentration within the tested range, was the main factor promoting phenolic extraction. In contrast, TF values were not significantly affected by solvent composition, despite slight variations in the mean values. HPLC-DAD chromatograms acquired at both 280 and 320 nm showed highly comparable qualitative profiles regardless of the extraction solvent, suggesting that solvent composition had little influence on the diversity of phenolic compounds extracted. Nevertheless, noticeable differences in peak intensity were observed among extracts. Hydroethanolic systems generally exhibited higher chromatographic responses, whereas the extract obtained with absolute ethanol presented lower signal intensities for several peaks, suggesting a lower extraction efficiency for some more polar phenolic constituents. The observed chromatographic profiles were consistent with the phenolic composition previously described for *C. monogyna* berries, supporting the suitability of hydroethanolic systems for recovering their major bioactive constituents [1,2]. The chromatographic results therefore complement the spectrophotometric analyses, indicating that solvent composition primarily affects extraction efficiency rather than the qualitative phenolic profile.

Overall, the incorporation of ethanol substantially enhanced the extraction of phenolic compounds compared with water extraction. Although no significant differences were detected among the hydroethanolic mixtures evaluated, their chromatographic profiles indicate a more efficient recovery of phenolic constituents in ethanol-water rather than in absolute ethanol, supporting the use of hydroethanolic mixtures as suitable extraction media for obtaining phenolic-rich *C. monogyna* berry extracts, with 75% ethanol resulting in an extract with a cleaner chromatographic baseline, suggesting a purer extract with fewer interfering compounds . These findings contribute to the optimization of extraction protocols and provide useful information for the development of natural antioxidant ingredients with potential applications in the food and nutraceutical industries.

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Bioaccessibility of Rice Bran Bioactive Compounds Following in Vitro Gastrointestinal Digestion

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Rice bran is a nutrient-rich by-product of rice milling and an important source of bioactive compounds, including ferulic acid (FA) and *p*-coumaric acid (*p*-CA), the major hydroxycinnamic acids in rice and among the most abundant phenolic acids in the rice bran layer. However, the physiological benefits of these compounds depend not only on their concentration in the food matrix but also on their bioaccessibility after gastrointestinal digestion. This study evaluated the effect of simulated gastrointestinal digestion on these compounds as well as their bioaccessibility in two cooked rice bran varieties (Ariete and Type III) using the standardized INFOGEST 2.0 static *in vitro* simulation of gastrointestinal food digestion protocol[1].

Cooked and lyophilized rice bran samples were subjected to simulated gastrointestinal digestion according to the INFOGEST 2.0 protocol. FA, *p*-CA, and total phenolic content (TPC) were quantified in the undigested samples and after oral, gastric and intestinal phases. FA and *p-CA* were determined by liquid chromatography coupled with mass spectrometry (LC–MS), whereas TPC was quantified by high-performance liquid chromatography with diode array detection (HPLC–DAD) at 280 nm. Bioaccessibility was expressed as the percentage of each compound recovered after digestion relative to its initial concentration in the undigested rice bran.

Results showed that FA and *p*-CA exhibited oral bioaccessibility of 35% and 60-65% for Type III and Ariete, respectively; however, their concentrations decreased markedly during the gastric and intestinal phases, resulting in intestinal bioaccessibility values below 2%. These findings suggest that gastrointestinal digestion substantially affects the stability of both compounds, likely due to chemical degradation, structural transformation, or interactions with the food matrix under digestive conditions. In contrast, TPC remained comparatively stable throughout digestion, with intestinal bioaccessibility ranging from 72 to 82%, indicating that a considerable proportion of the total phenolic fraction remained bioaccessible. The reduction in FA and p-CA observed during digestion is consistent with previous reports showing that digestive enzymes play a greater role than pH in modulating FA release, and structural modifications of hydroxycinnamic acids can occur throughout digestion[2]. Furthermore, similar observations were reported in sorghum bran, where *p-CA* became undetectable after intestinal digestion, while TPC remained stable, suggesting that changes in individual phenolic acids do not necessarily reflect the behaviour of the overall phenolic fraction[3].

Overall, simulated gastrointestinal digestion differentially modulated the phenolic profile of rice bran. While FA and *p*-CA showed markedly reduced intestinal bioaccessibility, the high recovery of TPC suggests that other phenolic constituents remain bioaccessible after *in vitro* digestion. These findings provide valuable information for understanding of the digestive fate of rice bran phenolic compounds and provide a foundation for future studies investigating their bioavailability and physiological relevance.

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Factorial Design Optimization of Natural Biopolymer-Based Emulsions for the Co-Encapsulation of Vitamins A and E

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Nutritional deficiencies remain a major concern in many populations, and lipophilic vitamin fortification has been explored as a strategy to alleviate this issue. Nevertheless, the high sensitivity of vitamins A and E to processing and storage conditions compromises their stability when incorporated directly into food matrices. In response to these limitations, emulsion-based encapsulation systems have gained attention as delivery approaches capable of protecting such bioactive compounds while sustaining their functional performance.[1] In this study, we aimed to develop and optimize physically stable oil-in-water emulsions for the simultaneous encapsulation of retinyl palmitate (vitamin A) and α-tocopheryl acetate (vitamin E).

A factorial design was applied to twelve formulations to assess the effect of three biopolymers, namely pea protein, gelatin, and sodium alginate, together with two emulsifiers, tween 80 and soy lecithin, and the presence or absence of calcium chloride as a crosslinking agent on emulsion stability. Sunflower oil was used as the lipid phase in all formulations, which were further coated with a 0.2% chitosan solution to improve resistance under acidic conditions.[2] The emulsions were characterized in terms of particle size, zeta potential and sedimentation index. Physical stability was also examined through a qualitative visual scoring system developed for this study and by accelerated storage testing under alternating temperatures (4 °C/40 °C) over seven days.[3]

The formulation composition significantly influenced microcapsule suspension stability. Among the tested systems, sodium alginate-based emulsions stabilized with soy lecithin and produced without calcium chloride showed the best performance, with lower sedimentation, favourable colloidal properties, and no significant phase separation under the conditions studied. These results indicate that sodium alginate combined with soy lecithin provides effective stabilization without ionic crosslinking. The optimized formulation appears to be a promising encapsulation system for the incorporation of vitamins A and E into fortified foods, supporting the development of functional ingredients with improved physical stability and potential use in bakery applications.

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Exploring the Nutritional Quality and Antioxidant Potential of Grass Pea, Chickpea and Lupin Flours

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The crop grass pea (*Lathyrus sativus* L.), chickpea (*Cicer arietinum* L.), and lupin (*Lupinus albus* L.) are pulse crops that provide numerous agronomic, nutritional, and environmental benefits. Their cultivation may help reduce hunger, improve human health, and mitigate soil degradation, benefits that align with the United Nations 2030 Agenda for Sustainable Development and the Sustainable Development Goals.

Grass pea, chickpea, and white lupin are annual grain legumes originating from Southern Europe and Western Asia[1], the Fertile Crescent[2], and the Mediterranean region[3], respectively. These crops are widely cultivated worldwide because they adapt to different environmental conditions. They are used for both human consumption and animal feed, as valuable sources of plant protein and dietary fibre.

In Portugal, these pulses are traditionally consumed in large quantities as side dishes and appetisers, usually after soaking and boiling to reduce toxic compound content. Further characterisation of nutritional and bioactive compounds is essential to support their incorporation into functional food products and to understand and clarify their potential health-promoting properties.

The nutritional composition and bioactive compounds of three legume flours - grass pea, chickpea, and lupin (**Figure 1**)- were evaluated. The grass pea and chickpea flours were pre-processed flours supplied by Egocultum (Santarém, Portugal), and the lupin flour was processed in the lab from lupin seeds that were soaked in water, boiled, dried, and milled. All legume flours were sieved to obtain particles smaller than 500 µm.



Lathyrus sativus L. *Cicer arietinum* L. *Lupinus albus* L.

Figure 1 – The proteinaceous pulse crops: grass pea (Lathyrus sativus L.), chickpea (Cicer arietinum L.), and lupin (Lupinus albus L.).

Protein content was determined by the Kjeldahl method, fat by Soxhlet extraction, and moisture and ash by gravimetric methods. Soluble and insoluble dietary fibres were quantified using an enzymatic/gravimetric method. Sugar, organic acid, and phenolic compound profiles were analysed by high-performance liquid chromatography (HPLC) using a refractive index detector for sugars and a photodiode array detector for organic acids and phenolic compounds. Total phenolic content was measured using the Folin–Ciocalteu assay, while antioxidant capacity was assessed using the DPPH and FRAP assays. All analyses were performed in triplicate.

Chickpea flour showed the greatest antioxidant capacity (1.53 ± 0.07 mmol TE/100 g by the DPPH method and 11.94 ± 1.56 mmol $FeSO_4$E/100 g by the FRAP method), and total phenolic compounds content (8.55 ± 1.51 mg GAE/100 g by the Folin–Ciocalteu assay and 196.48 ± 13.68 mg/100 g by the sum of each phenolic compound quantified by HPLC). Chickpea flour also had the highest total free sugar content (8.19 ± 1.05%) based on the sum of individual sugars quantified by HPLC.

Meanwhile, lupin flour had the highest contents of protein (28.14 ± 0.06%), fat (8.39 ± 0.03%), total organic acids (5.26 ± 0.72% by HPLC individual organic acid content sum), and dietary fibre (45.20 ± 0.96%), making it the most caloric (490 ± 2 kcal/100 g) of the studied pulse flours, despite its lowest total sugar content.

Regarding the phenolic compound profile, chickpea flour showed hydroxycinnamic (chlorogenic, p-coumaric and ferulic) and hydroxybenzoic (vanillic and syringic) acids, procyanidin, catechin, rutin, naringin, apigenin and quercetin as the main

phenolic compounds. Grass pea flour contained hydroxycinnamic (caffeic, p-coumaric and ferulic) and hydroxybenzoic (protocatechuic, vanillic and syringic) acids, procyanidin, catechin, rutin, naringin and apigenin. In contrast, lupin flour showed caffeic and vanillic acids, rutin and myricetin as the main phenolic compounds. All legume flours contained resveratrol.

The functionality of the 3 different legume flours was evaluated. Chickpea flour stood out for its balanced and adequate technological performance because of its water absorption capacity, water retention, and foaming and emulsifying properties.

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Physical quality and fungal incidence of chestnuts (Castanea sativa Mill.) under controlled atmospheres

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Postharvest losses in chestnuts (*Castanea sativa* Mill.) are largely driven by rapid physicochemical deterioration and fungal development, particularly under uncontrolled storage conditions. While controlled atmosphere (CA) storage has been proposed as a strategy to extend shelf life, the kinetic evolution of physical quality indicators and fungal incidence across a wide range of gaseous compositions remains insufficiently characterised. This study monitored the weekly evolution of key physical and microbiological quality parameters in Judia variety chestnuts stored under 14 controlled atmosphere conditions and two controls (positive: conventional refrigeration; negative: ambient atmosphere) over a 160-day period. The experimental design followed a Mixture Design approach with three gaseous components (O_2, CO_2 and N_2), applied in 32 duplicate chambers. Weekly assessments included mass loss and moisture content by gravimetry, water activity (Aw) by capacitance sensor, texture by Texture Profile Analysis (TPA), and colour parameters (L*, a*, b*) determined by two complementary methods: instrumental colorimetry (Konica Minolta) and digital image processing with Python algorithms applied to standardised photographic records (DSLR Nikon D5300, 24.2 MP) captured in a LED-illuminated cabinet to ensure consistent lighting conditions. The same image-based pipeline enabled automated detection and quantification of fungal and larval contamination across all experimental units throughout the storage period. Preliminary results up to week 11 show a progressive and time-dependent increase in fungal incidence, markedly more pronounced in negative control chambers compared to CA conditions, suggesting an inhibitory effect of modified gaseous environments on microbial development. Mass loss declined consistently across most CA treatments, while negative controls exhibited contrasting behaviour. Moisture content followed a similar trend, decreasing in the majority of CA conditions. Colour parameters and textural attributes also showed measurable changes over time, with the image-based colour analysis providing spatially resolved data complementary to point-based colorimetric readings. At this stage, storage duration appears to be the dominant factor driving quality changes, with differentiation between gaseous treatments expected to become more pronounced as the study progresses toward 160 days. Completion of the full dataset will enable statistical optimisation via Stat-Ease 22.0 to identify CA compositions that best preserve the physical integrity of chestnuts during extended storage.

Acknowledgements: This work was carried out under Agenda VIIAFOOD – Plataforma de Valorização, Industrialização e Inovação Comercial para o Agro-Alimentar (n.º C644929456-00000040), a project supported under the PRR (www.recuperarportugal.gov.pt), and financed by the European Union/Next Generation EU. This work was supported by national funds through FCT/MCTES (PIDDAC): CIMO, UID/00690/2025 (10.54499/UID/00690/2025) and UID/PRR/00690/2025 (10.54499/UID/PRR/00690/2025); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020). National funding by FCT, through the institutional scientific employment program contract with L. Barros (CEEC-INST, DOI: 10.54499/CEECINST/00107/2021/CP2793/CT0002) and A. Fernandes (FCT-TENURE: 2023.11031.TENURE.014). B. Melgar, T. Finimundy and N. Fernandes are grateful to the VIIAFOOD project (PRR Aviso n.º de agenda: C644929456-00000040) for their contracts.

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Nutritional and Sensory Characterisation of Traditional Gluten-Free Maize Muffins

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Maize is one of the world's most widely produced and consumed cereals, with an important role in human nutrition and the food industry. Growing demand for gluten-free products has also encouraged the development of formulations suitable for people with coeliac disease and those following gluten-free diets.

The aim of this study was to develop and perform the nutritional and sensory characterisation of gluten-free muffins made from a traditional Portuguese maize variety.

Two gluten-free carrot muffin formulations, with and without chocolate chips, were developed using flour from the traditional Portuguese maize variety 'Verdial'. The analytical methods used to determine the proximate composition were based on official AOAC (Association of Official Analytical Chemists) procedures. Moisture, ash, total lipid, crude fibre and crude protein contents were determined using methods 930.04, 930.05, 930.09, 930.10 and 978.04, respectively, with a nitrogen conversion factor of 6.25.[1] The results, excluding moisture content, were determined and expressed on a dry matter basis. A Welch's independent-samples *t*-test was used to compare the mean values obtained for the two muffin formulations. Statistical significance was established at $p < 0.05$.

Sensory evaluation sessions were conducted with potential consumers to assess sensory acceptance and purchase intention of the developed products. Sensory acceptance was evaluated using a nine-point hedonic scale, ranging from 1 'dislike extremely' to 9 'like extremely', to assess the attributes of appearance, aroma, crumb softness, flavour, and overall liking. The Acceptability Index (AI) was calculated to facilitate data interpretation. According to commonly adopted criteria in sensory studies, AI values ≥ 70% indicate good consumer acceptance and suggest that a product has potential for commercialisation.[2] Purchase intention was assessed using a five-point structured Purchase Intent Scale, ranging from 'Definitely would not buy' to 'Definitely would buy'.[3]

Analytical results showed that the muffins without chocolate chips had significantly higher moisture (22.77 ± 0.20 g/100 g) and ash (1.57 ± 0.02 g/100 g) contents than the formulation containing chocolate chips, which presented values of 16.11 ± 0.29 g/100 g and 1.49 ± 0.03 g/100 g, respectively. Regarding fat and crude fibre contents, the muffins without chocolate chips exhibited slightly lower mean values (9.83 ± 0.40 and 2.48 ± 0.14 g/100 g, respectively) than the chocolate chip formulation (9.87 ± 0.32 and 2.51 ± 0.14 g/100 g, respectively), although these differences were not statistically significant. In terms of protein content, the muffins containing chocolate chips showed a significantly higher value (5.58 ± 0.10 g/100 g) than the formulation without chocolate chips (5.24 ± 0.07 g/100 g). The nitrogen-free extract (NFE), which mainly reflects the available carbohydrate content, was similar between the muffins without chocolate chips (80.88 ± 0.47 g/100 g) and those containing chocolate chips (80.55 ± 0.45 g/100 g), with no statistically significant difference.

The acceptance and purchase intention tests were conducted with 51 potential consumers (56.9% female and 43.1% male). Participants were aged between 18 and 72 years, with a mean age of 28.3 ± 13.7 years. Muffins without chocolate chips achieved a higher purchase intention (60.7%) than muffins with chocolate chips (33.3%), indicating a clear difference in consumer purchase intention between the two formulations. For all sensory attributes evaluated, the muffins without chocolate chips achieved mean Acceptability Index (AI) values above 70% and consistently higher than those obtained for the chocolate chip formulation. The overall liking AI reached 78.6% for the muffins without chocolate chips, compared with 69.7% for the chocolate chip muffins. These findings were consistent with the purchase intention results, as the muffins without chocolate chips showed a substantially higher purchase intention and the highest average sensory acceptability scores. This suggests that higher sensory acceptability, particularly overall liking, positively influenced consumers' willingness to purchase the product. This study contributes to the valorisation of traditional ingredients by promoting the use of the Portuguese traditional 'Verdial' maize variety in the development of innovative gluten-free products. This approach also supports value creation by providing a new application and potential market opportunity for a traditional maize variety.

Acknowledgements: We would like to thank all the volunteers who participated in the sensory evaluation.
Funding: This work was supported by CERNAS (UID/00681/2025; https://doi.org/10.54499/UID/00681/2025) through the FCT – Foundation for Science and Technology, I.P., and by the European Union (Grant No. 101059872), the Swiss State Secretariat for Education, Research and Innovation (Contract No. 22.0412) and UK Research and Innovation (UKRI).

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Nutritional Characterisation and Sensory Acceptance of Gluten-Free Muffins Made with Portuguese 'Verdial' Maize Landrace Flour

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Maize is one of the world's most widely produced and consumed cereals, with an important role in human nutrition and the food industry. Growing demand for gluten-free products has also encouraged the development of formulations suitable for people with coeliac disease and those following gluten-free diets.

The aim of this study was to develop gluten-free muffins using flour from the Portuguese 'Verdial' maize landrace and to evaluate their nutritional composition, sensory acceptance and purchase intention.

Two gluten-free carrot muffin formulations, with and without chocolate buttons, were developed using flour from the traditional Portuguese maize variety 'Verdial'. The analytical methods used to determine the proximate composition were based on official AOAC (Association of Official Analytical Chemists) procedures. Moisture, ash, total lipid, crude fibre and crude protein contents were determined using methods 930.04, 930.05, 930.09, 930.10 and 978.04, respectively, with the latter calculated using a nitrogen conversion factor of 6.25.[1] The results, excluding moisture content, were expressed on a dry matter basis. A Welch's independent-samples *t*-test was used to compare the mean values obtained for the two muffin formulations. Statistical significance was established at $p < 0.05$.

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Physicochemical, microbiological and sensory evaluation of vacuum-packaged fresh marinated beef

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Meat quality is largely judged through sensory attributes such as color, aroma, texture and flavor, which directly determine consumer acceptance. Marination by vacuum tumbling is a simple industrial operation with the potential to upgrade lean cuts of low commercial value, but its combined effect on quality, stability and safety during refrigerated storage is still poorly documented. This study, carried out at Carnes Campicarn, S.A., aimed to evaluate the influence of two marinade formulations on the physicochemical, microbiological and sensory characteristics of vacuum-packaged beef over 14 days of refrigerated storage.

Commercial eye of round cuts (n = 25) from cattle older than 24 months were used - a lean cut frequently associated with lower tenderness and prone to color changes. Samples were assigned to three treatments: control (no marinade), marinade F (a simple formulation based on bell pepper and onion) and marinade D (a more complex formulation). Cuts were sliced to 8 mm and 25 mm thickness, marinades were incorporated in a vacuum tumbler for 5 min, and the samples were vacuum-packaged and stored at 4 °C for 14 days. The physicochemical parameters assessed were pH, water activity (aw), moisture (NP 1614-1:2009), fat (NP 1613:1979), cooking loss, CIE L*a*b* color, Warner–Bratzler shear force and lipid oxidation (TBARS). Microbiological quality was assessed by the enumeration of microorganisms at 30 °C (ISO 4833-1:2013/Amd 1:2022), *Enterobacteriaceae* (ISO 21528-2:2017), *Listeria monocytogenes* (ISO 11290-2:2017), *Escherichia coli* (ISO 16649-2:2001) and detection of *Salmonella spp.* (ISO 6579-1:2017/Amd 1:2020). Acceptability of the marinated samples was assessed in a consumer test conducted at the company with 35 panelists familiar with this type of product. Data were analyzed by two-way ANOVA (product × storage time) followed by Tukey's HSD test ($p < 0.05$), after verification of normality and homoscedasticity; the joint structure of the physicochemical data was explored by principal component analysis (PCA) on standardized variables.

The marinades significantly affected beef quality. Marinade F showed the lowest cooking loss (24.28%), indicating greater water-holding capacity, together with lower shear work (28.35 N·mm) and firmness (8.45 N), indicating improved texture; its pH also decreased during storage, consistent with the activity of lactic acid bacteria. Both marinades caused significant changes ($p < 0.05$) in color, particularly in the a* and b* coordinates, possibly related to the spices in the formulations. Marinade D showed greater oxidative stability, with a lower TBARS value at day 14 (0.494 mg MDA/kg) than marinade F (1.060 mg MDA/kg). The chemical composition of the meat remained characteristic throughout storage, with water activity between 0.962 and 0.968, moisture between 73.77% and 75.29% and fat between 1.66% and 2.64%.

The microbiological counts remained within the acceptable limits for vacuum-packaged beef, demonstrating that the application of marinades did not compromise the microbiological quality and safety of the product. In the consumer test, Marinade F was the preferred sample (66%) and received the highest ratings on a hedonic scale for the attributes of texture, color, aroma, overall appreciation and flavor intensity.

The PCA (Figure 1), computed on nine standardized physicochemical variables, retained 62.1% of the total variance. PC1 (45.1%) opposed samples with higher aw, pH, L*, firmness and shear work (control, and marinade D at day 0) to marinade F, characterized by higher a*, b*, moisture and TBARS, whereas PC2 (17.0%) was associated with storage time in the marinated products, the 14-day F and D samples being displaced towards the TBARS vector, a trajectory not followed by the control. Because the scores of both marinades drifted along PC1 during storage, the separation between products on this axis is partly confounded with storage time. The partial overlap of the control and marinade D ellipses nevertheless indicates a profile closer to that of unmarinated meat, whereas the displaced marinade F ellipse reflects its distinct color, softer texture and higher oxidation.

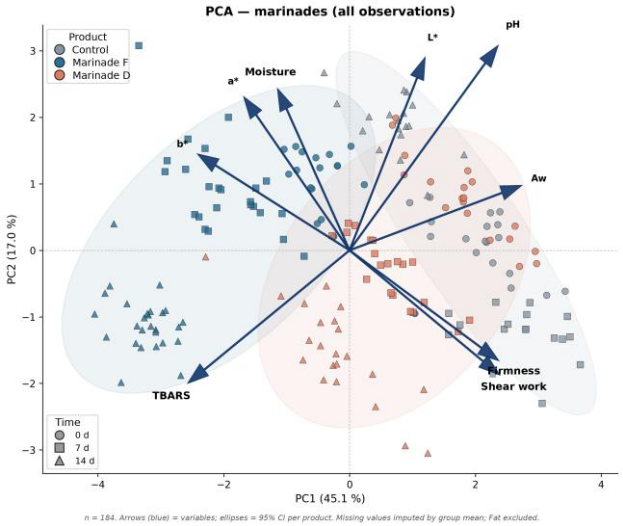


Figure 1 – PCA biplot of the physicochemical data (all observations).

Microbiological counts remained within the acceptable limits for vacuum-packaged beef throughout storage, showing that the marinades did not compromise the microbiological quality and safety of the product. In the consumer test, marinade F was the preferred sample (66%) and received the highest hedonic scores for texture, color, aroma, flavor intensity and overall acceptance.

Overall, marination by vacuum tumbling proved to be an effective technological strategy to add value to cuts of lower commercial value, improving quality attributes relevant to the consumer while preserving microbiological stability during refrigerated vacuum storage. The contrast between the two formulations also highlights a trade-off between sensory performance (marinade F) and oxidative stability (marinade D) that should guide formulation choices at industrial scale.

The results show that marinating processed by vacuum tumbling constitutes an effective technological strategy to add value to cuts with lower commercial value, improving quality characteristics relevant to the consumer and preserving microbiological stability during refrigerated vacuum storage.

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Development of a Synbiotic Biscuit: Evaluation of *Heyndrickxia* coagulans Stability and Prebiotic Substrates for Functional Bakery Applications

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The development of synbiotic bakery products represents a promising strategy to deliver health-promoting ingredients in convenient food matrices. This study aimed to evaluate the suitability of the probiotic *Heyndrickxia* coagulans (formerly *Bacillus* coagulans) and different prebiotic substrates for the development of a synbiotic biscuit with potential nutritional claims related to gut health and weight management.

Initially, the effect of the biscuit manufacturing process on the viability of H. coagulans was evaluated. The probiotic was cultivated in TGY (Tryptone–Glucose–Yeast Extract) medium, incorporated directly into the biscuit dough at approximately 1.23 x1010 CFU/g, and baked at 100 °C for 30 min. Viable cell counts were determined on TGY agar before and after baking and during storage at room temperature. Although baking reduced viable cell counts due to thermal stress, the remaining population remained stable throughout storage, suggesting that encapsulation is not required under the evaluated processing conditions (Figure 1).

To identify a suitable prebiotic ingredient for the synbiotic biscuit formulation, the growth of H. coagulans was evaluated in MRS broth in which glucose was replaced by different prebiotic substrates as the sole carbon source[1]. The tested substrates included commercial inulins and fructooligosaccharides (FOS) with different degrees of polymerization, together with in-house-produced FOS obtained from sucrose and carob pulp. Probiotic growth was monitored by optical density measurements.

The results demonstrated that all tested prebiotic substrates supported the growth of H. coagulans, confirming their ability to serve as carbon sources and their suitability for application in synbiotic formulations. These findings provide the basis for selecting appropriate prebiotic ingredients for the development of functional synbiotic biscuits with potential health benefits.

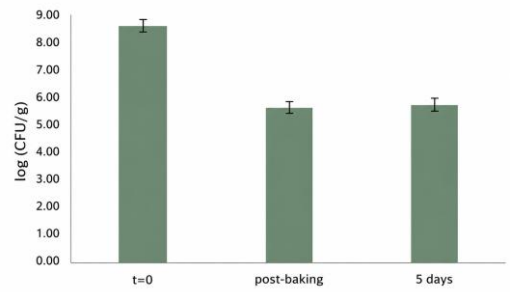


Figure 1 – Viability of the probiotic Bacillus coagulans during cookie processing and storage.

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Preserving Traditional Food Heritage through Nutritional Optimisation: A Case Study from the Gastronomic Heritage of the Coimbra Region

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Traditional foods represent an important component of cultural heritage, preserving local identity, culinary knowledge, and regional biodiversity. However, many traditional recipes were developed under historical dietary contexts that differ from current public health recommendations. Food chemistry provides valuable tools for understanding the physicochemical properties of ingredients and processing techniques, enabling nutritional optimisation while preserving the structural, sensory, and cultural characteristics that define traditional foods.

This work focused on the nutritional optimisation of fifteen traditional recipes documented in the *Carta Gastronómica da Região de Coimbra*[1], aiming to demonstrate how gastrotechnical principles and food science can contribute to the preservation and valorisation of Portuguese gastronomic heritage. Traditional dishes and desserts were analysed to identify opportunities for improving their nutritional profile without compromising authenticity, technological functionality, or sensory quality. The work was developed within the curricular unit Gastrotechnics and Experimental Dietetics of the Bachelor's Degree in Nutrition and Dietetics at the Coimbra Health School (ESTeSC), Polytechnic University of Coimbra, Portugal.

Recipe reformulation was guided by the physicochemical behaviour of food components during processing. Modifications included the replacement of fat sources to improve fatty acid composition, reduction of sodium and added sugars, incorporation of ingredients with improved nutritional value, optimisation of thermal processing, and adjustment of ingredient ratios to preserve texture, flavour, colour, moisture retention, and overall product stability[2]. The technological consequences of these interventions were considered to ensure that the defining characteristics of each traditional preparation were maintained.

The reformulated recipes demonstrated that evidence-based modifications founded on gastrotechnical principles and food science can successfully improve nutritional quality while preserving the gastronomic identity of traditional foods. Preparations such as *Feijoada de Sames* (Figueira da Foz), *Tiborna* (Tábua), and *Queijadas* (Pereira) illustrate how careful optimisation can reduce undesirable nutritional components without compromising the culinary attributes that contribute to their cultural significance.

These findings highlight the important role of food chemistry in safeguarding traditional food heritage. Understanding the molecular and technological mechanisms underlying ingredient functionality and culinary processes allows scientifically supported adaptations that respect authenticity while addressing contemporary nutritional challenges. This approach contributes to the sustainable preservation of regional gastronomy, reinforcing traditional foods as valuable elements of cultural identity and healthy dietary patterns.

Keywords: Traditional foods; Food heritage; Gastrotechnics and Experimental Dietetics; Nutritional optimisation; Gastronomic heritage.

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Uniformity of honey samples with PDO across NUTS III and harvest years

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Honey is a highly valued natural product known for its nutritional and bioactive benefits. Its composition is affected by various factors, especially the geographical origin and environmental conditions of where it is produced. These factors influence the honey's nutritional and sensory qualities. Protected Designation of Origin (PDO) certification plays a crucial role by ensuring the product's authenticity and maintaining its regional identity, which in turn boosts the economic value of certified honeys. This study examined several physicochemical parameters of honey samples across different regions with DOP, aggregated at the NUTS level III from the 'Terra Quente' PDO (Alto Tâmega e Barroso; Trás-os-Montes; Douro; Beiras e Serra da Estrela) in north-eastern Portugal, including acidity, pH, water content, water activity, electrical conductivity, total phenols and flavonoids, colour parameters in the CIELab system (L*, a*, b*), Pfund scale, 5-hidroximetilfurfural (HMF), proline, and ash content.

All the results obtained are within the legally established limits. The results show clear variability among the measured parameters. The Pfund colour ranged from 29 to 146 mm on the Pfund scale, the proline content from 126.44 to 931.43 mg/kg, the total phenolic content (TPC) from 40.18 to 124.30 mg GAE/100 g, and the total flavonoid content (TFC) from 1.30 to 6.93 mg QE/100 g.

The PCA represented in Figure 1 demonstrates a low level of variability among the samples, as the first two principal components explain only 56% of the total variability. This is a positive outcome because it reflects the expected uniformity within the PDO region. However, slight variability can be observed between NUTS III and across years, which may be due to minor regional differences in floral composition and climatic variations.

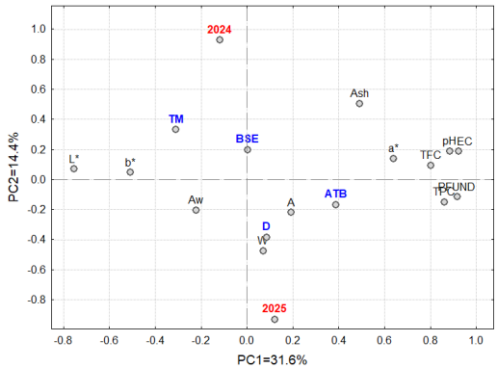


Figure 1 - Principal component analysis based on analytical parameters from 'Terra Quente' region with DOP. Ash: Ashes; A: Acidity; L*: Colour coordinate L*; a*: Colour coordinate a*; b*: Colour coordinate b*; W: Water content; Aw: Water activity; EC: Electrical conductivity; TPC: Total phenolic content; TFC: Total flavonoids content; PFUND: Pfund scale; TM: Trás-os-Montes; BSE: Beiras e Serra da Estrela; D: Douro; ATB: Alto Tâmega e Barroso.

The results confirm the quality of honey produced under the 'Terra Quente' PDO, as well as the homogeneity of samples collected from different regions within the PDO. This supports their certification. The small differences detected among NUTS III and between years are associated with local flora and annual climatic conditions rather than indicating substantial regional heterogeneity.

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"Vinegar Mothers" Derived from Surplus Food Resources as a Source of Cellulose-Producing Acetic Acid Bacteria

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Vinegar production involves two successive biological processes: alcoholic fermentation, during which yeasts convert sugars into ethanol and CO_2, followed by acetification, in which obligately aerobic acetic acid bacteria (AAB) incompletely oxidize ethanol into acetic acid. Acetification can be performed using traditional French acetifiers (Orleans method), German rapid acetifiers, or submerged culture systems. In the Orleans process, AAB develop at the liquid-air interface, forming the so-called "vinegar mother", a biofilm composed of bacterial cells embedded in extracellular biopolymers, predominantly bacterial cellulose (BC). BC, a biopolymer produced by AAB, has attracted considerable attention due to its outstanding mechanical strength, high water-holding capacity, and excellent biocompatibility, often surpassing the properties of plant-derived cellulose. These characteristics make BC a promising biomaterial for a wide range of industrial, biomedical, and environmental applications. The aim of the present study was to isolate AAB from "vinegar mothers" and screen their capacity to produce BC. "Vinegar mothers" were collected from artisanal vinegar productions carried out using the Orleans method with three different fermentation substrates: surplus pomegranates, surplus grapes, and organoleptically altered beer. They were supplied by a company located in the Algarve region (Portugal). A total of 21 bacterial isolates were recovered, purified on Hestrin-Schramm (HS) agar medium at 30 °C, and identified by phylogenetic analysis of the 16S rRNA gene sequence. Bacterial cellulose production was evaluated in liquid HS medium (pH 5.5) supplemented with glucose, in static cultures, and incubated for two weeks at 25 °C. Cellulose biofilms formed at the air-liquid interface were harvested, washed with distilled water at 100 °C, treated with 0.1 M NaOH at 80 °C, neutralized with distilled water, and freeze-dried. BC mass was estimated by determining the dry weight of the purified dried biofilms. The 21 isolates were identified as members of the genera *Acetobacter* and *Komagataeibacter*. Of these, 15 isolates were able to produce bacterial cellulose under the conditions tested. The highest production was observed among isolates obtained from grape "vinegar mothers", belonging to the genus *Komagataeibacter*, with biofilm dry weights ranging from 0.33±0.20 to 0.43±0.039 g/L. Future work will focus on optimizing culture conditions to enhance cellulose yield and on physicochemical characterization of the produced bacterial cellulose. Additionally, the potential use of low-cost substrates derived from agro-industrial by-products will be investigated to improve the sustainability and economic feasibility of bacterial cellulose production.

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VALORIZAÇÃO ETNOBOTÂNICA DE DUAS ESPÉCIES MEDICINAIS DO NORTE DE ANGOLA: PERFIL FITOQUÍMICO E POTENCIAL FUNCIONAL ALIMENTAR

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As províncias do Bengo e do Uíge, no norte de Angola, conservam um conhecimento etnobotânico associado ao uso tradicional de plantas medicinais. Deste modo, foram selecionadas duas espécies, *Ochna afzelii* e *Steganotaenia araliacea*, cuja caracterização no contexto angolano e potencial como fontes de compostos bioativos para aplicações alimentares permanecem pouco explorados. O objetivo deste estudo foi caracterizar o perfil fitoquímico, à atividade antioxidante, a inibição da oxidação lipídica e a atividade antibacteriana dos seus extratos. As folhas destas plantas foram higienizadas, desidratadas e trituradas, sendo preparados extratos aquosos. O teor de compostos fenólicos totais foi analisado pelo método Folin-Ciocalteu, a atividade antioxidante pelos métodos DPPH, FRAP e ABTS[1, 2] a capacidade de inibição da oxidação lipídica pelo ensaio TBARS e a atividade antibacteriana pela determinação dos halos de inibição a Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli B e E. coli O157[3].

O. afzelii apresentou um teor de fenois totais de 4,74 mg/g de planta seca, com valores de TEAC para DPPH, FRAP e ABTS de 11,40, 158,2, 2745,8 µmol Trolox/g de planta seca, respetivamente. Por outro lado, *S. araliacea* apresentou um teor de fenóis totais de 2,40 mg/g de planta seca, com valores de DPPH, FRAP e ABTS de 22,76, 104,5, 2717,1 µmol Trolox/g de planta seca, respetivamente. No ensaio TBARs, *S. araliacea* apresentou EC50 de 6,0 µg/mL, comparativamente a 8,0 µg/mL, para *O. afzelii*. A atividade antibacteriana foi limitada nas condições testadas, não tendo sido observados halos de inibição relevantes para os extratos de nenhuma das espécies. A análise fitoquímica do extrato *O. afzelii*, permitiu identificar Apigenina-C-acetilhexosideo, crisoeiol-8-C-hexosideo-6-Cpentosideo, ambos numa concentração individual de 2,8±0,1 mg/g de extrato seco; No extrato de S. araliacea foram identificados ácido 3,5-di-O-cafeoilquínico (45±1 mg/g de extrato seco), ácido malonil di-O-cafeoilquínico (11,3 ± 0,3 mg/g de extrato seco) e quercetina-acetilhexosídeo (8,9 ± 0,3 mg/g de extrato seco).

Os resultados evidenciam a presença de compostos bioativos, destacando-se a *O. afzelii* pelo maior teor de fenóis totais; *S. araliacea* pela maior eficiência na inibição da oxidação lipídica e pela presença de elevados teores de derivados cafeoilquínicos.

As duas espécies apresentaram uma capacidade antioxidante semelhante, especialmente pelos métodos FRAP e ABTS. Estes dados contribuem para a valorização científica do conhecimento etnobotânico do Bengo e do Uíge e sugerem que O. *afzelii* e *S. araliacea* podem constituir fontes de compostos naturais de interesse para futuras aplicações alimentares, nomeadamente como ingredientes funcionais ou alternativas naturais a determinados aditivos sintéticos.

Palavras-chave: Etnobotânica; Plantas medicinais; Angola; Compostos fenólicos ; Atividade antioxidante; Ingredientes funcionais.

Agradecimentos: Os autores agradecem as comunidades locais do Bengo e do Uíge pela partilha do conhecimento etnobotânico que fundamentou a seleção das espécies estudadas.
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NMR-Metabolomics in Aquaculture - case studies from farmed fish and shrimp

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Nuclear Magnetic Resonance (NMR)-Metabolomics is a powerful approach to assess metabolome in several aquaculture species, providing an expeditious tool for metabolite profiling and to evaluate responses to various production conditions, such as nutritional or environmental challenges. It can also be applied to several different tissues (muscle, liver, intestine, plasma), while providing complementary information to other approaches (zootechnical performance, histology, enzymes, and other Omics). In recent years, we have applied NMR-Metabolomics in a spectrum of studies thus contributing to an integrated analysis of biological systems within real aquaculture production scenarios and challenges.

In all studies, well-established and consolidated metabolomics workflows were followed: tissue aqueous fraction extraction, NMR spectral acquisition, spectral processing, and data treatment by multivariate analysis. The NMR-Metabolomics approach was applied to: i) assess the effects of dietary glycerol supplementation on the muscle and liver metabolome of rainbow trout (Oncorhynchus mykiss) and European seabass (Dicentrarchus labrax), aiming to spare dietary protein for growth while utilizing a valuable biodiesel production by-product; ii) profile and evaluate the effects of a high digestible starch content diet on the muscle and liver metabolome of barramundi (Lates calcarifer) as a complementary approach to the assessment of the hepatic glycogen synthesis; iii) evaluate the metabolic effects of tributyrin supplementation (a triacylglyceride of short-chain fatty acids known to reduce the negative effects of high inclusion levels of plant protein) in rainbow trout (O. mykiss) fed a plant-based diet, combined with the hepatic and intestinal mRNA analysis to assess tissue-specific responses; iv) analyse the metabolomic profiles of plasma and digesta in 1 trait-selected along with 2 commercial strains of rainbow trout (O. mykiss) fed plant- or fishmeal-based diets to support histological data on the onset of enteritis.

More innovative technical / methodological approaches were taken in the development of NMR-based high-throughput automated routines for systematic monitoring of fish plasma profile in aquaculture research and industrial quality control analysis (Tavares et al. 2022), as well as the in the use of direct and intact (non-extracted) use of muscle fish tissue by HRMAS (high-resolution magic angle spinning) NMR (Jarak et al. 2019). As worldwide aquaculture aims to diversify target species and consumers shift to different aquatic protein sources, a new focus has been directed at crustacean species, namely the giant tiger prawn (Penaeus monodon) and locally sourced brown shrimp (Crangon crangon). Determination of the geographic origin and species of marine fish fillet can also benefit from the development of NMR-based analyses (Palma et al. 2026) for authenticity, traceability and carbon footprint.

NMR-based metabolomics proved to be a suitable tool for contributing to more sustainable and resilient aquaculture production systems thus raising the bar on seafood quality and profiling.

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Physicochemical, Nutritional, and Functional Characterization of Myrtle (*Myrtus communis L.*): Berries, Leaves and Liqueur

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Myrtus communis L. is a Mediterranean plant (Figure 1) with potential for food and nutraceutical applications due to its nutritional value and content of bioactive compounds. This study aimed to characterize the physicochemical, nutritional and functional properties of myrtle berries and leaves, comparing samples from wild and cultivated plants, as well as leaves at different developmental stages and from different harvesting locations. Biometric and physicochemical parameters, nutritional and mineral composition, reducing sugars, extraction yield, total phenolic and flavonoid contents, and antioxidant activity were evaluated using DPPH and ABTS assays. The physicochemical characteristics of myrtle liqueur were also assessed.



Figure 1 – Myrtle berries and leaves.

Wild berries showed higher compression resistance (12.5 N), soluble solids content (24.80 °Brix), dietary fibre content (18.76 g/100 g), magnesium (12.16 mg/100 g DW) and potassium (61.69 mg/100 g DW), as well as higher reducing sugar content and extraction yield (66.45%). In contrast, cultivated berries presented larger dimensions, higher moisture (41.32 g/100 g), ash content (4.02 g/100 g), phosphorus concentration (1.357 mg/100 g DW), and total phenolic content (708.89 mg GAE/L). Wild leaves showed higher flavonoid content (187.07 mg QE/L) and DPPH antioxidant activity (1231.95 mg TE/L), whereas cultivated leaves showed higher ABTS antioxidant activity. Young leaves presented a higher total phenolic content (1060.49 mg GAE/L) than older leaves. The myrtle liqueur showed 42 °Brix, pH 4.6, and titratable acidity of 0.98 g/L and 0.83 g/L, expressed as tartaric and citric acid, respectively. Overall, the results indicate that plant origin and leaf developmental stage influence the physicochemical composition and bioactive potential of M. communis, supporting its potential as a source of functional compounds for food and nutraceutical applications[2].

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Diplotaxis tenuifolia as a Potential Health-Promoting Food: Nutritional, Phytochemical, and Antioxidant Properties

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The growing interest in sustainable and nutrient-rich plant resources has encouraged the exploration of cultivated species with valuable nutritional and bioactive properties. *Diplotaxis tenuifolia* (Brassicaceae), commonly known as wild rocket, is valued for its culinary use and recognized as a source of bioactive compounds, particularly glucosinolates and phenolic compounds. However, its comprehensive nutritional composition, broader phytochemical profile, and overall bioactive potential remain insufficiently characterised, particularly regarding less-studied secondary metabolites such as oxylipins. This study evaluated the nutritional composition, phytochemical profile, and antioxidant activity of *D. tenuifolia* cultivated in Portugal.

Certified seeds of *Diplotaxis tenuifolia* (L.) *DC*. were obtained from Saflax (Lübeck, Germany) and cultivated. Leaves were harvested in June 2024, prior to flowering. Nutritional and food-safety analyses were performed according to official AOAC International methods, including the determination of moisture, ash, lipids, protein, fibre, and minerals. Mineral elements were quantified by flame atomic absorption spectrometry (FAAS), phosphorus by spectrophotometry, and lead and cadmium by graphite furnace atomic absorption spectrometry (GFAAS), while mercury was determined by direct thermal combustion. Cellulose, hemicellulose, and lignin were calculated from neutral detergent fibre, acid detergent fibre, and acid detergent lignin, respectively, using the Van Soest method. Total amino acid content was analysed by UPLC-PDA following acid hydrolysis, and fatty acid profile was determined by GC-FID after conversion to fatty acid methyl esters (FAMEs). Five extracts were prepared from lyophilized leaves using 80% methanol by reflux at 70 °C for 6 h, 100% and 50% (v/v) ethanol and water by maceration for 6 h, and water by infusion for 15 min. Total phenolic and flavonoid contents were determined using the Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively, and antioxidant activity was assessed by ABTS, DPPH, and FRAP assays. The extract showing the highest phenolic and flavonoid contents and antioxidant activity was further characterised by HPLC-PDA-ESI-MSn.

Calcium, potassium, magnesium, iron, manganese, and chromium provided more than 15% of their respective Nutrient Reference Values (NRVs) in the raw material, meeting the criterion for a significant amount according to Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011. The levels of lead and cadmium were below the maximum levels established for leafy brassica vegetables under Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food. The amino acid profile showed glutamic and aspartic acids as the most abundant amino acids, followed by leucine, lysine, and valine. The fatty acid profile was dominated by unsaturated fatty acids, with α-linolenic acid (C18:3 n-3) as the major fatty acid. The 80% methanol reflux extract showed the highest total phenolic and flavonoid contents, with values of 125.41 ± 0.92 mg gallic acid equivalents g^{-1} extract and 3.72 ± 0.06 mg quercetin equivalents g^{-1} extract, respectively, and the highest antioxidant activity, with 39.43 ± 0.97 and 12.96 ± 0.02 mg trolox equivalents g^{-1} extract for ABTS and DPPH, respectively, and 752.71 ± 4.59 µmol Fe(II) g^{-1} extract for FRAP. HPLC-PDA-ESI-MSn analysis enabled the characterisation of 20 compounds, including phenolic acids, flavonol glycosides, oxylipins, and glucosinolates, based on MS^n fragmentation patterns and comparison with literature data. Sulfoglucobrassicin was identified for the first time in *D. tenuifolia*. Other compounds identified included 6-(methylsulfonyl)-3-oxohexyl glucosinolate, proline, pipecolic acid, glucaric acid, arachidic acid (C20:0), 9,10,12,13-tetrahydroxyoctadecanoic acid (sativic acid), and 9,12,13-trihydroxyoctadec-10-enoic acid, which, as far as we know, were reported for the first time in *D. tenuifolia* leaves.

Overall, these findings highlight *D. tenuifolia* as a nutritionally rich edible plant and a promising source of natural antioxidant and bioactive compounds, supporting its potential for functional food and nutraceutical applications. Further studies are warranted to elucidate the biological effects of its individual constituents and the factors influencing their composition and concentration.

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XVIII Encontro de Química dos Alimentos

Coimbra | 14 - 16 de outubro'26

COMUNICAÇÃO DE CIÊNCIA – QUÍMICA ALIMENTAR, LITERACIA ALIMENTAR E ESCOLHA DO CONSUMIDOR



Carlos Fiolhais

Professor catedrático jubilado da Universidade de Coimbra

Professor Catedrático Emérito de Física da Universidade de Coimbra. Doutorado em Física Teórica pela Universidade Goethe, em 1982, foi fundador e diretor do Centro de Física Computacional da Universidade de Coimbra, diretor da Biblioteca Geral da Universidade e fundador do Rómulo – Centro Ciência Viva da Universidade de Coimbra. Autor de mais de 150 artigos científicos, desenvolve também uma reconhecida atividade de comunicação e divulgação de ciência, sendo autor de mais de 500 artigos de carácter pedagógico e de divulgação. Entre os seus contributos para a ciência em Portugal destaca-se a instalação do primeiro supercomputador no país e a publicação da primeira História da Ciência em Portugal. Ao longo da sua carreira recebeu diversas distinções, entre as quais a Ordem do Infante D. Henrique e o Globo de Ouro de Ciência da SIC, ambos em 2005.

Física e Alimentos no Dia Mundial da Alimentação: da Termodinâmica na cozinha às alterações climáticas globais

Fiolhais, C.

Professor emérito de Física da Universidade de Coimbra e membro do Centro de Estudos Globais da Universidade Aberta

O Dia Mundial da Alimentação, que se celebra mundialmente a 16 de Outubro no dia da fundação da FAO, organização das Nações Unidas para a Alimentação e Agricultura (a criação foi 16 de Outubro de 1945 em Québec, Canadá), tem, em 2026, como lema "Inovar hoje para nutrir o amanhã" como tópicos a inovação no sistema agroalimentar, a resiliência climática e a acessibilidade económica para reduzir a fome no globo. A física sempre contribuiu para o progresso do conhecimento da alimentação humana, designadamente no processo da sua preparação e conservação: basta lembrar que contabilizamos a energia dos alimentos em calorias, em consequência de avanços feitos na termodinâmica no início do século XIX: uma caloria é a energia necessária para elevar de 1 ºC a temperatura de um quilograma de água [1]. A introdução dos frigoríficos, dos fornos de micro-ondas e dos fogões de indução na cozinha no século XX foram momentos em que a física esteve presente para facilitar a vida doméstica. No plano da restauração, a moderna gastronomia molecular é o resultado das ciências físico-químicas: foi o investigador francês Hervé This (n. 1955) é um investigador físico-químico francês, a trabalhar no Instituto Nacional de Pesquisa na Agricultura, Alimentação e Ambiente, em Versailles- Saclay, que conjuntamente com o físico húngaro-britânico Nicholas Kurti (1908–1998), professor da Universidade de Oxford, criou essa disciplina do final dos anos de 1980 [2]. A gastronomia molecular estuda os mecanismos físicos e químicos nos processos de confecção de alimentos.

Mas um contributo assaz relevante da física para a alimentação encontra.se também na questão das alterações climáticas, que se deveram a avanços na Revolução Industrial, que está diagnosticada pela ciência, existindo «terapias» eficazes a que chamamos energias alternativas. . De facto, ao aquecimento global, que os físicos do clima têm estudado como no quadro da termodinâmica e da mecânica dos fluidos, tem colocado sérios constrangimentos à produção alimentar e ao funcionamento das cadeias de fornecimento (3). Para os enfrentar, inovações recentes, como o uso de *drones* na vigilância de culturas agrícolas, sensores da Internet das Coisas para rega mais eficaz, e Inteligência Artificial generativa para optimizar produção e distribuição , na área da física e engenharia, e a agricultura regenerativa, a moderna edição genética (CRISP) e novas fontes de proteínas (por exemplo, carne artificial), na área da biologia e da bioquímica, estão a contribuir para resolver o magno problema de fornecer as calorias indispensáveis à vida de todos os seres humanos no mundo. A fome no mundo infelizmente ainda existe, mas trata-se mais de um problema socioeconómico e político do que de um problema científico-tecnológico. E o mesmo se pode dizer do mundo previsivelmente mais povoado ao longo do resto do século XXI. A ciência dá conhecimento, a tecnologia soluções, mas é, afinal a sociedade, que tem de tratar do seu futuro sustentável.

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Daniel Silva é Business & Dissemination Manager do GreenCoLab – Associação Oceano Verde, onde desenvolve atividade nas áreas da biotecnologia, inovação e valorização de recursos algais. Licenciado em Biotecnologia pela Universidade da Beira Interior e mestre em Biotecnologia Alimentar pela Universidade de Aveiro, possui ainda uma pós-graduação em Gestão de Marketing pela Porto Business School. Ao longo do seu percurso profissional, adquiriu experiência em diferentes setores da indústria alimentar, tendo integrado, antes de ingressar no GreenCoLab, a Allmicroalgae – Natural Products, onde participou no desenvolvimento das áreas de comunicação e marketing, bem como na gestão comercial de produtos alimentares. Desde 2020, no GreenCoLab, coordena a comunicação institucional e operações internas, contribuindo para a disseminação e valorização de soluções baseadas em algas. Tem participado em iniciativas nacionais e internacionais relacionadas com biotecnologia azul, inovação alimentar, sustentabilidade e economia azul.



Hugo Pereira é Coordenador Geral do GreenCoLab – Laboratório Colaborativo de Tecnologias e Produtos do Oceano Verde. Com mais de 15 anos de experiência em biotecnologia de algas, tem trabalhado ao longo de quase toda a cadeia de valor, da bioprospeção ao desenvolvimento de novos produtos para diversas aplicações biotecnológicas. Antes de assumir funções no GreenCoLab, foi investigador no grupo MarBiotech do Centro de Ciências do Mar (CCMAR) e integrou os departamentos de I&D da Necton S.A. (Belamandil) e da Allmicroalgae (Pataias). É autor de mais de 100 publicações científicas e de 4 capítulos de livros, e tem apresentado o seu trabalho em diversas conferências internacionais.



Margarida Martins é Doutorada em Engenharia Química, com background em Biotecnologia Alimentar, e possui mais de 10 anos de experiência na área das algas e da biotecnologia marinha. Atualmente, é responsável de inovação na ALGAplus, onde coordena a gestão de projetos, o desenvolvimento de novos produtos e a identificação de oportunidades de financiamento. Promove a colaboração entre investigação e indústria e desenvolve atividades de comunicação e divulgação científica junto da população e de diferentes stakeholders, contribuindo para aproximar o conhecimento científico, o setor empresarial e a sociedade.

The Whole Ocean on a Plate – Science, Cuisine and Algae Production

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The Whole Ocean on a Plate - Science, Cuisine and Algae Production is a multidisciplinary book developed to bridge scientific knowledge, algal production, and culinary innovation, highlighting the potential of microalgae and macroalgae as emerging resources for sustainable food systems and the blue bioeconomy, (**Figure 1**). The book follows the journey of algae from production and valorisation to their application in food and gastronomy, providing an integrated perspective on their diversity, characteristics, history, potential, and technological applications.



Figure 1 – Book "The Whole Ocean on a Plate"

Developed through a collaboration between GreenCoLab – Associação Oceano Verde and PROALGA – Associação Portuguesa dos Produtores de Algas, the publication brings together expertise from three complementary perspectives within the Portuguese algae sector: scientific research, industrial production, and gastronomy, represented by Hugo Pereira (GreenCoLab), João Navalho (Necton), and chef Leonel Pereira (CHECKin Restaurant), respectively. Their contributions are complemented by those of researchers and specialists from academic, research, and industry institutions, including Instituto Superior de Agronomia (ISA), Laboratório Nacional de Energia e Geologia (LNEG), Centro de Ciências do Mar do Algarve (CCMAR), Necton, ALGAplus, and GreenCoLab.

Beyond its scientific and technical content, the publication places strong emphasis on visual communication. Its high-quality graphic design and extensive photographic work by Jorge Simão, a Portuguese award-winning photojournalist and specialist in food photography, complement the scientific and culinary content and contribute to translating the diversity and potential of algae into an accessible visual narrative.

Overall, the publication presents algae not only as biological and technological resources, but also as an interface between science, innovation, production, culture, and gastronomy. By bringing together these different perspectives in a single publication, *The Whole Ocean on a Plate* demonstrates the potential of interdisciplinary collaboration to promote knowledge transfer, stimulate innovation, and strengthen the role of algae in the Portuguese and European blue bioeconomy.

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Consultor, investigador, docente e designer nas áreas do Food Design e da Gastronomia, desenvolvendo atividade na interseção entre design, sistemas alimentares, hospitalidade, cultura e experiência. Doutorado em Design pela Faculdade de Arquitetura da Universidade de Lisboa, é Professor Auxiliar na Universidade Europeia, onde desenvolve atividade científica e pedagógica nas áreas do Food Design, gastronomia criativa, inovação em hospitalidade, turismo, sustentabilidade e sistemas alimentares. A sua investigação aborda a alimentação enquanto ferramenta de conhecimento, memória, identidade, inovação e valorização cultural. Colabora com universidades, instituições culturais, organizações de turismo, marcas de hospitalidade, restaurantes e equipas criativas, participando no desenvolvimento de experiências gastronómicas, programas de inovação, estratégias territoriais e conceitos de restauração sensorial e sustentável.

Food Design Atlas: Os Mapas Invisíveis dos Sistemas Alimentares

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A comunicação '**Food Design Atlas: Os Mapas Invisíveis dos Sistemas Alimentares**' propõe uma abordagem ao food design como prática de mediação crítica entre a ciência alimentar, a literacia e a educação alimentares e as escolhas do consumidor. Partindo do pressuposto que os sistemas alimentares são estruturas complexas, constituídas por inúmeras *layers* frequentemente invisíveis — entre as quais os processos químicos dos alimentos, as transformações sensoriais, as cadeias de valor, os impactos ambientais, as práticas culturais, as memórias, os hábitos e os fatores que condicionam a tomada de decisão —, a comunicação explora de que modo o food design pode tornar estas relações mais legíveis, experienciáveis e culturalmente significativas.

Ao recorrer à metáfora do atlas, a comunicação estrutura-se como uma cartografia das dimensões invisíveis que atravessam o alimento, da química da matéria alimentar à perceção sensorial, da literacia e da educação alimentares à complexidade dos sistemas de produção, distribuição e consumo. Neste contexto, o food design é entendido não apenas como o "desenho" de produtos, serviços ou experiências, mas também como uma abordagem transdisciplinar capaz de traduzir conhecimento científico em dispositivos sensoriais, narrativos, pedagógicos e participativos, promovendo processos de colaboração e cocriação entre os diferentes *stakeholders* dos sistemas alimentares.

A comunicação incide igualmente sobre o papel da experiência alimentar na construção e na apropriação do conhecimento, na formação de consumidores mais críticos e na promoção de escolhas alimentares informadas, conscientes e sustentáveis. Com base na investigação e na prática aplicada nos domínios da gastronomia participativa, da educação em food design, da inovação alimentar e da mediação cultural através do alimento, propõe-se que comunicar a ciência dos alimentos implica mais do que transmitir informação, mas também criar as condições necessárias para que essa informação seja compreendida, experienciada, questionada e incorporada nas práticas alimentares quotidianas.

Influence of front-of-package labelling on behavioral, nutritional, and cognitive-affective outcomes regarding food and beverages marketed to children

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Early childhood is a critical window for establishing dietary patterns and shaping health trajectory. Front-of-package nutritional labels(FOPL) have emerged as a policy tool designed to aid consumers make healthier food choices. However, FOPL effectiveness for foods and beverages marketed to children remains uncertain. This systematic review followed the PRISMA 2020 and a registered protocol (PROSPERO CRD420251117263). Five databases and BASE were searched between January 2014 and July 2025. Eligible studies assessed summary, nutrient-specific and mixed FOPL formats for packaged foods and beverages selected for children aged 0-12 years and/or their caregivers. Risk of bias was assessed using JBI tools, and certainty of evidence using GRADE for quantitative data and GRADE-CERQual for qualitative data. Findings were synthesized by direction of effect, sign-test, and narratively.

The review included thirty-nine studies, of which 18 were randomized controlled trials, 5 were quasi-experimental, 6 analytical cross-sectional, and 10 qualitative studies. Evidence suggests that the effects are more consistent in perceived healthfulness and intention-related outcomes. Overall, the FOPL reported a favorable effect on different outcomes, notably product purchase, product selection, or perceived healthfulness. However, nutritional-profile outcomes were more heterogenous and, for that, less consistent, being often supported with low or very low certainty of evidence. Warning labels showed a clearer direction pattern, particularly by reduction of selection or intention to purchase less healthy products. Across various outcomes, educational material tended to favor the presence of FOPL; however, evidence was insufficient to demonstrate a clear benefit. Qualitative findings suggested that FOPL can function as tools that help caregivers select healthier products, albeit their use is shaped by other factors such as trust, comprehension and context.

FOPL may aid consumers in judging healthfulness and purchase intention, but sustained nutritional impact remains uncertain. None of the FOPL types or formats arose as universally superior, thus policies should focus on matching label formats to specific goals and populations.

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One Health and Food Chemistry: Literacy, Sustainability and Integrated Risk Management in Food Systems

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Early childhood is a critical window for establishing dietary patterns and shaping health trajectory. Front-of-package nutritional labels(FOPL) have emerged as a policy tool designed to aid consumers make healthier food choices. However, FOPL effectiveness for foods and beverages marketed to children remains uncertain. This systematic review followed the PRISMA 2020 and a registered protocol (PROSPERO CRD420251117263). Five databases and BASE were searched between Introduction: Food systems face growing challenges arising from the interdependence of human health, animal health and environmental sustainability. In this context, the One Health approach provides an integrated framework for understanding food-related risks, promoting safer choices and strengthening sustainability throughout the stages from primary production to consumption (Silva et al., 2022).

Food Chemistry plays a central role at this interface by supporting the understanding of food composition, contaminants, nutritional quality, the effects of processing, authenticity and safety. These dimensions are directly related to food literacy, consumer behaviour and the development of more sustainable food systems.

Objectives: To critically reflect on the contribution of Food Chemistry to the One Health approach, with particular emphasis on food literacy, food sustainability and integrated risk management. To explore how chemical knowledge can support informed decisions regarding food safety, responsible consumption, environmental protection and public health.

Methods: A narrative review with technical and scientific reflection was conducted, based on searches of the Scopus and Web of Science databases covering the period 2020–2025 and focusing on One Health, food literacy, sustainability and integrated risk management. The analysis considered the interdependence between the One Health approach, the Sustainable Development Goals, Environmental, Social and Governance principles, the European Green Deal, Political, Economic, Social, Technological, Environmental and Legal analysis, and risk management perspectives.

Results: The approaches analysed highlight the importance of interdisciplinary and collaborative approaches to addressing complex challenges related to food systems, climate change, antimicrobial resistance, environmental degradation, technological innovation and consumer awareness. The analysis suggests that Food Chemistry can act as a bridge between scientific knowledge and practical decision-making within food systems.

Chemical literacy applied to food can improve understanding of additives, contaminants, allergens, nutrition claims, food preservation, packaging, authenticity and environmental impacts. From a One Health perspective, food literacy is not limited to individual food choices; it also involves understanding how food production, processing, transport, consumption and waste affect ecosystems, animal welfare and public health. Integrating Food Chemistry into One Health education can therefore strengthen critical thinking, promote safer food practices and encourage more sustainable consumption patterns.

The One Health approach is aligned with the Sustainable Development Goals, particularly those related to good health and well-being, quality education, responsible consumption and production, climate action, life below water and life on land (Sachs et al., 2024; Silva et al., 2022). Its articulation with ESG principles and the European Green Deal reinforces the importance of governance, environmental responsibility, social impact and systemic risk management in food-related decision-making (Ahmad et al., 2023). In this context, tools such as PESTAL analysis and cause-and-effect diagrams can support the identification of risks and opportunities across political, economic, social, technological, environmental and legal dimensions.

Conclusion: The integration of One Health, Food Chemistry, Food Literacy and Food Sustainability provides a relevant framework for promoting safer, healthier and more responsible food systems. Although this study does not present original empirical data, it provides a technical and scientific reflection on the role of chemical knowledge in supporting informed food choices, sustainability strategies and interdisciplinary education. Strengthening food literacy through a One Health perspective may contribute to more resilient communities and to a more sustainable relationship between humans, animals and the environment.

Keywords: One Health; food chemistry; food literacy; food sustainability; integrated risk management.

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From canned fish chemistry to label design: science communication and food literacy in schools

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Food literacy and science communication are crucial for promoting informed, critical, and responsible food choices, particularly among young consumers. Everyday foods are effective educational tools because they connect chemistry with gastronomic tradition, technology, safety, sustainability, and consumer choices. In this context, canned fish is especially suitable due to its cultural and economic relevance in Portugal and its potential to illustrate key concepts in food chemistry and food labeling.

This work presents the initiative “The Chemistry behind Canned Fish”, promoted by the Division of Education and Dissemination of Chemistry of the Portuguese Chemical Society, in partnership with Instituto Superior de Engenharia do Porto (ISEP), Polytechnic of Porto, and Cofisa, a canned fish industry partner. Developed within the celebrations of the National Day of Portuguese Canned Fish, celebrated annually on November 15 since 2022, the activity aimed to demystify chemistry through a familiar food product and to bring students closer to analytical and food chemistry. The initiative was structured into two sequential phases: a first phase dedicated to workshops on the chemistry of canned fish, held in schools and at ISEP, and a second phase consisting of a national label design contest.

The first phase involved 503 lower-secondary and secondary students from five schools in the Porto metropolitan area and was implemented in two complementary formats. In the school-based sessions, students watched an introductory video on the history of canned fish and the industrial canning process, from fish capture and processing to sterilization and packaging. This was followed by a presentation on food labels and the information they provide to consumers. Students also participated in a comparative tasting of canned tuna in oil and in brine, which promoted discussion of ingredients, formulation, nutritional composition, and consumer perception. Additional activities addressed the presence of microplastics in seafood and label analyses and included interactive resources, such as questionnaires and word-search exercises, to reinforce concepts related to label interpretation. Students who visited ISEP followed the same introductory structure, including the video, the presentation on food labels, and the comparative tasting of the two canned tuna products. They then took part in laboratory-based activities, observing how nutrients declared on food labels can be analytically determined and how microplastics in seafood can be analyzed. This component linked labeling information with experimental evidence and highlighted the role of analytical chemistry in food quality, safety, and authenticity.

Following the engagement generated by these activities, a national label design contest was launched, challenging students to apply chemistry knowledge, creativity, and communication skills to develop innovative and informative canned fish labels. The first edition exceeded expectations, receiving 308 submissions from 415 students, with support from 50 teachers and participation from 98 schools; 250 works were submitted by lower-secondary students and 58 by secondary students. The quality and creativity of the proposals demonstrated that food labels can strengthen the connection among science, Portuguese food heritage, and marine environmental awareness.

Overall, “The Chemistry behind Canned Fish” demonstrated the impact of using traditional foods as vehicles for science communication. By integrating historical context, hands-on activities, laboratory work, and a label design contest, the initiative promoted food literacy, student engagement, and awareness of chemistry's role in everyday food choices, seafood quality, and ocean sustainability..

Acknowledgments: This work received financial support from Portuguese national funds (FCT/MECI, Fundação para a Ciência e a Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (DOI: 10.54499/UID/50006/2025). The authors gratefully acknowledge Cofisa S.A. for kindly supplying the canned tuna in oil and brine used during the science communication activity “The Chemistry behind Canned Fish”. Manuela M. Moreira also thank FCT/MCTES for funding through the Scientific Employment Stimulus - Individual Call (2023.05993.CEECIND/CP2842/CT0009,DOI:10.54499/2023.05993.CEECIND/CP2842/CT0009).
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Biological and Behavioral effects of sucralose and erythritol in *D. melanogaster*

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Erythritol (E968) is a naturally occurring polyol, industrially produced via glucose fermentation, with about 60–80% of sucrose's sweetness, very low caloric value, and high stability. It is largely absorbed in the small intestine and excreted unchanged in urine, leading to its long-standing safety approval. However, recent studies indicate it can trigger biological responses in various organisms, prompting renewed interest in its physiological effects. Sucralose (E955) is a synthetic sweetener about 600 times sweeter than sucrose. Although approved for consumption, it persists in the environment and, upon thermal or metabolic degradation, yields hazardous byproducts like 6-acetyl-sucralose and 6-chlorofructose, which are clastogenic and compromise DNA integrity. Recent research also links sucralose to long-term toxicity risks and oxidative stress.

The fruit fly D. melanogaster is a key model organism due to its short life cycle, high fecundity, low maintenance cost, and genetic homology with humans, facilitating multi-generational studies. Erythritol exposure in Drosophila reduces adult survival, impairs reproduction, and increases larval mortality, showing insecticidal effects. Similarly, sucralose impacts survival, reproduction, and locomotor behavior. While individual effects of these sweeteners are documented, studies on their combined exposure remain limited, with strong evidence that erythritol may potentiate sucralose-induced oxidative stress. This work therefore aimed to assess the biological and behavioral effects of combined erythritol and sucralose exposure in *D. melanogaster*, evaluating DNA integrity and locomotor behavior to better understand the interactions of these commonly co-consumed sweeteners.

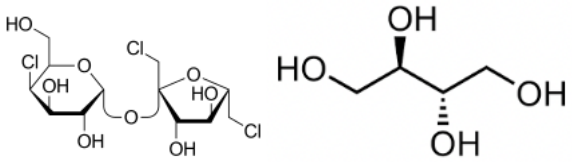


Figure 1 - Erythritol and sucralose

The study demonstrates that combined exposure to erythritol and sucralose induces significant genotoxic and neurotoxic effects in Drosophila melanogaster. In the comet assay, sucralose alone caused considerable DNA fragmentation, and the addition of erythritol exacerbated this damage in a dose-dependent manner, suggesting erythritol potentiates the oxidative stress generated by sucralose. The locomotor performance assay (negative geotaxis) showed a parallel decline, with flies taking progressively longer to climb after exposure to the sweetener mixture, indicating functional neurological impairment. This synergistic toxicity highlights that two commonly consumed and individually approved sweeteners can become harmful when combined, primarily through the promotion and enhancement of oxidative stress.

These findings have important public health implications, as they underscore the need to evaluate chemical mixtures in risk assessment, rather than only individual compounds. The observed dose-dependent effects and the potential saturation at higher doses point to a complex toxicological interaction. Future research should extend to vertebrate models and human cell cultures to better extrapolate the risks to human health and to investigate broader physiological impacts, such as longevity and reproduction, which were not addressed in this study.

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Impact of Industrial-Scale Pulsed Electric Fields on Olive Oil Quality: A Case Study from a Portuguese Farmers' Cooperative

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Extra virgin olive oil is one of Portugal's most important agri-food products, with the cultivars Cobrançosa and Galega representing the main varieties used for olive oil production. In recent years, there has been growing interest in the application of non-thermal technologies capable of improving extraction efficiency without compromising olive oil quality[1]. Among these, pulsed electric fields (PEF) have emerged as a promising technology by promoting cell membrane electroporation and facilitating oil release[2,3]. However, information on its application at the industrial scale remains limited. Therefore, the present study aimed to evaluate the application of PEF technology in a cooperative olive mill located in Murça (Northern Portugal), using olives from the Cobrançosa and Cordovilcultivars. Industrial extraction tests were conducted on olive mash was subjected to two different PEF modalities across distinct assays pre-malaxation, allowing a comparison between control, monopolar PEF, and bipolar PEF-treated groups. The selected electric field strength was 2 kV/cm, with a specific energy of 5.5 kJ/kg. Square wave pulses of 30 µs were applied for the monopolar protocols, whereas the bipolar protocols used +15 / -15 µs pulses, with a relaxation time of 50 to 70 µs between polarities. The frequency was adjusted according to the flow rate and pulse current to consistently achieve the target specific energy. The resulting olive oils were analysed for quality parameters, including free acidity, peroxide value, and specific extinction coefficients (K232 and K268). Total phenolic content, antioxidant activity (DPPH assay), oxidative stability, pigment content (carotenoids and chlorophylls), and colour parameters were also determined. A techno-economic analysis was conducted to estimate the operational cost of the PEF process, considering the specific energy input (kJ/kg) and electricity consumption (kWh/t of olive paste). The results obtained under these conditions demonstrate that PEF-assisted extraction could improve the oil yield by up to 17%, according to the results obtained through the determination of percentage residual oil in the pomace. The application of PEF to olive paste did not significantly affect the quality parameters of the olive oils, and all samples complied with the legal requirements for the extra virgin olive oil category. Peroxide value showed a decreasing trend, from 8.10 meq O_2/kg in the control to 6.64 and 6.12 meq O_2/kg in the monopolar and bipolar PEF treatments, respectively.

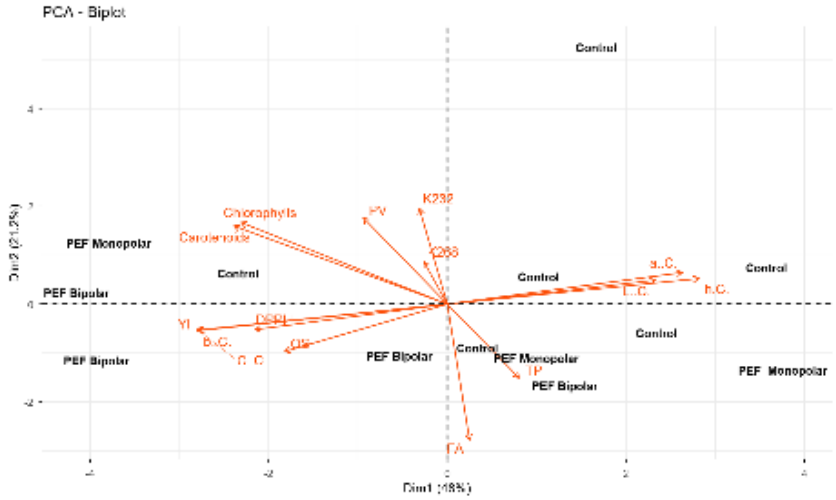


Figure 1 - Loading plots and PCA score for differentiation of control and PEF-assisted extraction with monopolar and bipolar conditions, based on quality parameters of the olive oils.

Regarding the bioactive compounds, total phenolic content averaged 508.56 mg GAE/kg in the control, 497.85 mg GAE/kg in the monopolar PEF treatment, and 530.24 mg GAE/kg in the bipolar PEF treatment, whereas antioxidant activity (DPPH) ranged from 42.22% to 47.11%. Given the specific energy input of 5.5 kJ/kg, and since PEF *operational expenditure* (OPEX) consists almost exclusively of electricity costs (1.53 kWh/t), the calculated cost is €0.32 per tonne of processed olive oil mash. PCA explained 69.2% of the total variability and highlighted the separation between treatments and production days,

beginning and end of November, (***Figure 1)***. PC1 was mainly associated with colour coordinates, photosynthetic pigments (chlorophylls and carotenoids), and antioxidant activity (DPPH), whereas PC2 was primarily driven by free fatty acidity (FA), K232, peroxide value (PV), total phenolic content (TP) and pigment composition, indicating that both harvest period and PEF treatment could has influenced the physicochemical profile of the olive oils. These findings demonstrate that PEF can be applied without compromising the physicochemical and functional properties of the extra virgin olive oil, supporting its potential as a promising technology for improving the extraction process, provided that future studies confirm its impact on extraction efficiency and industrial yield.These findings demonstrate that PEF can be applied without compromising the physicochemical and functional properties of the extra virgin olive oil, supporting its potential as a promising technology for improving the extraction process, provided that future studies confirm its impact on extraction efficiency and industrial yield.

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Institutionalising Food Safety: Ferreira da Silva, Charles Lepierre and the Emergence of Official Analytical Methods in Portugal, 1895–1945

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The introduction of official procedures for food analysis in Portugal coincided with the increasing involvement of chemical laboratories in food control activities. Such a connection made chemical analysis relevant to the state's interests in inspection, enforcement, and standardization. In this communication, the role of the *Comissão Técnica dos Métodos Químico-Analíticos* in the development and standardization of chemical and regulatory analysis methods in Portugal will be discussed, with particular attention to the contributions of António Joaquim Ferreira da Silva (1853-1923) and Charles Lepierre (1867-1945).

This institution was founded in 1895 as the Commission for Standardizing the Analytical Methods of Wines, Olive Oils, and Vinegar (Ferreira da Silva) due to disagreements over the procedure for the salicylation of Portuguese wines exported to Brazil. In 1904, the entity was transformed into the Commission for Technical Methods of Chemical Analyses and expanded to other laboratories of state institutions. Its structure was officially recognized in the Decree dated 27 June 1918, when renowned chemists Ferreira da Silva, Álvaro Basto, Luís Rebelo da Silva, Aquiles Machado, Charles Lepierre, and João Holtreman de Faria came together.

Ferreira da Silva and Lepierre's careers illustrate two different perspectives on the development of the institutionalization of analytical chemistry in the context of food[3] (**Figure 1**). Ferreira da Silva, who was the director of the Municipal Chemical Laboratory of Porto (LQMP) and the president of the Commission, has helped make analysis an official procedure, thereby making the results of chemists' work more legally and administratively valid. The latter was a professor at the Instituto Superior Técnico (IST) and, since 1935, the director of the laboratory at the Portuguese Institute for Fish Canning[1,2]. Lepierre represents a later stage in which analytical chemistry focused on a wider spectrum of products and industries. Between 1933 and 1945, Lepierre analysed canned sardines, lead pollution, protein nitrogen destruction, marine fish oils, milk, and milk flours.

Drawing on legislation, materials concerning Charles Lepierre at the IST, studies and publications concerning Ferreira da Silva and the LQMP of the Porto Municipality, and papers published in *Revista de Chimica Pura e Applicada*, this communication demonstrates that standardized methods of analysis emerged through sustained institutional collaboration. It also shows that the recognition of analytical chemistry as a form of expertise was indispensable to the development and implementation of public food safety policies (which would not have been possible without it). Using our methodological tools for the institutionalization of Portuguese chemistry, this communication will also reveal how the emergence of analytical chemistry became central to Portuguese food regulation and remains important to current debates on scientific authority.

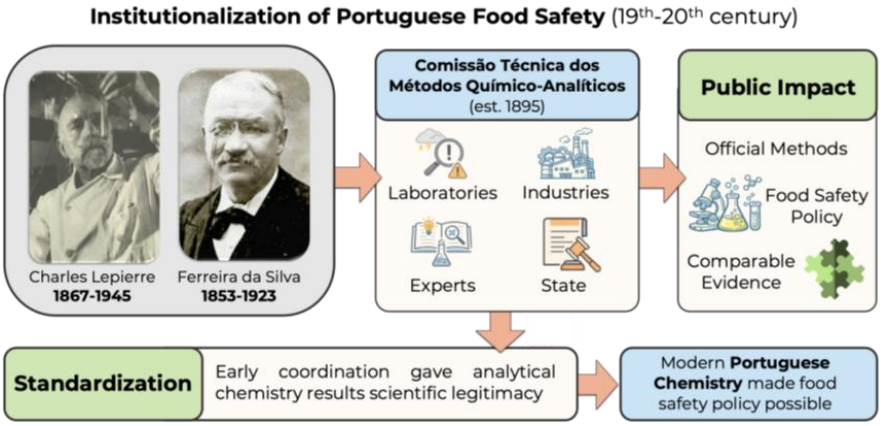


Figure 1 – Schematic overview of the Institutionalization of Portuguese Food Safety though the emergence of analytical chemistry.

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ScreenBETES: AI Platform for Antidiabetic Bioactive Discovery from Natural Products

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Type 2 diabetes affects more than 537 million adults worldwide, and its multi-target pathophysiology has driven the search for new bioactives from natural products and foods. However, this discovery is constrained by the cost, throughput, and resource consumption of experimental assays. Because plant and food extracts are complex mixtures containing dozens to hundreds of metabolites, identifying which compound is responsible for the activity usually requires bioassay-guided fractionation, an iterative process that multiplies the number of tests needed and, with them, the consumption of solvents, reagents, and plant biomass, as well as laboratory waste. To overcome these limitations, we developed ScreenBETES (Figure 1), a freely accessible artificial intelligence platform that predicts activity against 17 validated antidiabetic targets (including SGLT2, PPARγ, PTP1B, DPP-4, and α-glucosidases) directly from chemical structure. The quantitative structure–activity relationship (QSAR) models were built on curated ChEMBL data following field best practices [1], with model selection based on the Matthews correlation coefficient (MCC) and validation through external holdout, Y-scrambling, and explicit definition of the applicability domain. The final models achieved MCCs of 0.71 to 0.95 and AUC-ROC values of 0.92 to 0.99, with each prediction accompanied by an applicability-domain assessment that flags unreliable extrapolations. Thanks to this approach, the platform screens more than 100,000 compounds in under 30 minutes, and allows prioritizing. Application to the LOTUS database (276,518 natural compounds) [2] illustrates this potential: ScreenBETES prioritised 7,395 high-confidence candidates, of which about 25% comply with Lipinski's rule of five, and independent literature confirmed predictions for food-derived compounds, including aspalathin from rooibos (*Aspalathus linearis*) and structurally related C-glucosyl dihydrochalcones reported as selective SGLT2 inhibitors [3], demonstrating prospective validation on structures absent from the training set. ScreenBETES may help reduce the experimental effort, time, and resource footprint of bioactive discovery, contributing to a more sustainable and economically viable valorization of food bioactives as antidiabetic nutraceutical candidates. The work thus aligns with the United Nations Sustainable Development Goals, in particular SDG 3 (Good Health and Well-being), SDG 9 (Industry, Innovation and Infrastructure), and SDG 12 (Responsible Consumption and Production).

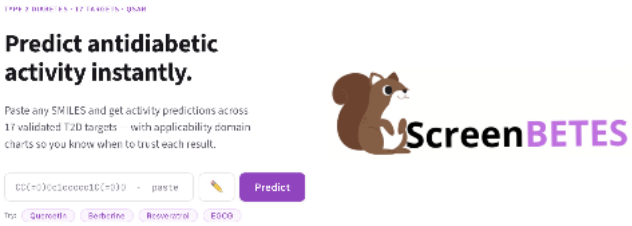


Figure 1 - ScreenBETES platform interface: the user enters a chemical structure (SMILES) and gets predictions of antidiabetic activity in 17 validated type 2 diabetes targets, with applicability domain graphs to assess the reliability of each result.

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Hyperspectral Imaging Study for Identification of Meat Species

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The global growing consumption of meat, combined with the growing concern with product authenticity and quality control, has reinforced the need for fast, reliable and non-destructive analytical methods.[1,2] Hyperspectral imaging (HSI) has emerged as a promising technology in the agri-food sector, by combining spatial and spectral information that allows a detailed characterization of food products.[3] This study aimed to evaluate the potential of hyperspectral imaging in the identification and discrimination of different meat species, steer and chicken, as well as hamburgers produced from them and made with mixtures of beef and chicken in different proportions (Beef blend burger/ Chicken 75%/25% (H75) e Beef blend Burger/ Chicken 50%/50% (H50)). Additionally, the relationship between spectral data and physicochemical parameters was investigated. Conventional physicochemical analyses were carried out to determine the contents of moisture, protein, total fat, pH, total pigments and color parameters (CIELab). Hyperspectral images were acquired using two hyperspectral cameras, in the VIS-NIR (400-1000 nm) and NIR (900-1700 nm) spectral ranges.

The results showed that beef and chicken meat have distinct chemical compositions that directly influence the absorption of radiation in the VIS-NIR and NIR regions, reflected in their own spectral signatures, Figure 1. The obtained spectra were analyzed in detail, and the absorption regions associated with the different constituents, such as water, fat, protein and total pigments, were successfully identified. Multivariate statistical methods were applied to the spectral data. Principal Component Analysis (PCA) was used in an exploratory way, from the data that allowed to observe a clear separation between the different meat species, both in whole meat samples and in hamburgers, although with less differentiation. The PCA revealed that much of the variance of the data can be explained by a few principal components, which reinforces the existence of specific spectral signatures for each type of meat and justifies the use of discriminative models for classification purposes for such classification.

The results obtained in this study demonstrate the ability of hyperspectral imaging (HSI) to effectively differentiate between beef and chicken, as well as to identify hamburgers produced from these species based on their spectral characteristics. The integration of HSI with chemometric techniques, namely Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), proved to be a powerful approach for the assessment of meat authenticity, enabling the discrimination of samples according to species and product type. The distinct spectral patterns observed among the analysed samples highlight the potential of this methodology for supporting food authentication and quality control applications.

Although the present work was conducted using a limited number of samples, the results consistently demonstrate the applicability of the proposed approach and provide a strong foundation for future research. Expanding the sample set to include a greater diversity of products, origins, and processing conditions would allow a broader representation of natural variability and further strengthen the robustness and transferability of the methodology.

Overall, these findings reinforce the value of hyperspectral imaging as a rapid, non-destructive, and non-invasive analytical tool for the food industry. Its capacity to acquire detailed spectral information in real time, combined with advanced multivariate analysis techniques, makes it particularly suitable for the detection of food fraud, including the mislabelling, substitution, or adulteration of meat products. Consequently, HSI represents a promising technology for enhancing quality assurance systems, improving traceability, and increasing consumer confidence throughout the food supply chain.

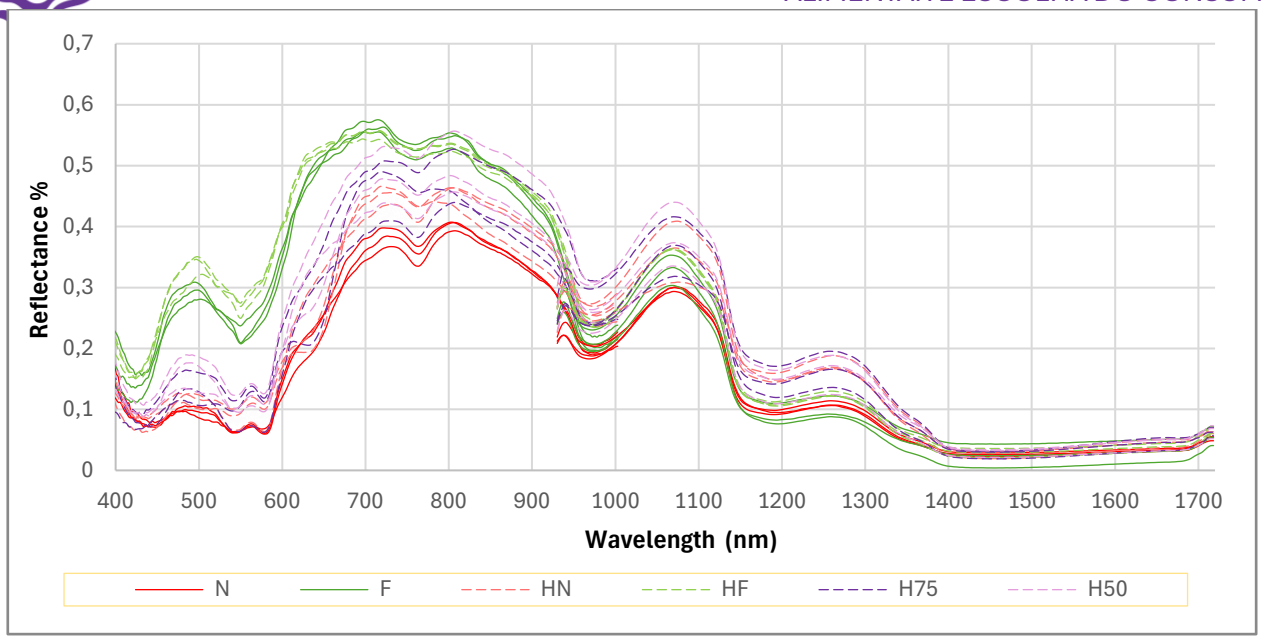


Figure 1 - Reflectance spectra of the Steer (N), Chicken (F), Beef Burger (HN), Chicken Burger (HF), Beef blend burger/ Chicken 75%/25% (H75) e Beef blend Burger/ Chicken 50%/50% (H50).

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Validation of a food literacy scale using structural equation modelling

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Healthy food literacy can be understood as the extent to which individuals are capable of accessing, interpreting, and applying basic nutritional information, as well as using available resources and services to make informed decisions that support their health. This concept goes beyond the simple acquisition of information, involving the transformation of knowledge into practical understanding that enables individuals to adopt appropriate dietary behaviours. Consequently, people with higher levels of food literacy are more likely to make conscious and informed food choices, contributing to improved health outcomes. Conversely, insufficient food literacy may lead to inadequate dietary decisions, which can negatively influence health and increase the risk of nutrition-related diseases. [1]

This study aimed to develop and validate a scale for assessing food literacy, and to investigate factors linked with food literacy, as represented by the conceptual model developed and shown in Figure 1.



Figure 1 – Conceptual model for assessing food literacy.

The validation process was carried out among 924 Portuguese university students. The initial version of the scale consisted of 50 items, which were analysed through exploratory factor analysis and structural equation modelling for confirmatory factor analysis. The final validated instrument was structured as a second-order model, including a general factor referred to as “Food literacy” and comprising 26 items grouped into three dimensions: F1—knowledge related to the nutritional composition of foods (10 items), F2—knowledge regarding food labelling and food selection (7 items), and F3—knowledge and practices associated with healthy eating behaviours (9 items). The scale demonstrated excellent internal consistency, with Cronbach’s alpha values above 0.9, and the Pearson correlations between the three dimensions and the overall construct were also higher than 0.9. Overall, the findings confirm the validity of this scale, supporting its use as a reliable tool for assessing food literacy.[2]

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Consumption of edible insects – a transnational study on their gastronomic use

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In a context of continuous global population growth, the demand for food is expected to increase substantially, with projections indicating an increase of approximately 50%. This scenario poses significant challenges to food security, encouraging the development of more sustainable, efficient, and resilient strategies to ensure future food availability. Within this framework, edible insects (EI) have emerged as a promising alternative to conventional animal-based protein sources, due to their high nutritional value and considerably lower environmental impact associated with their production. Several edible insect species exhibit a remarkable nutritional profile, particularly due to their high protein content and the presence of proteins with high biological value, containing a wide range of amino acids, including essential amino acids. In addition to proteins, EI are also valuable sources of lipids, especially polyunsaturated fatty acids, carbohydrates, and important micronutrients, such as vitamins and minerals. [2] Furthermore, edible insects contain bioactive compounds that may contribute to food techno-functionality and provide potential health benefits for humans. Among these compounds are phenolic compounds and dietary fibre, which have been associated with beneficial physiological effects. [3] Several biological activities have been reported for components derived from edible insects, including antioxidant, anti-inflammatory, antimicrobial, and antiviral effects, as well as potential protective effects against the development of metabolic syndrome-related disorders. [3]

This study evaluates consumer perceptions about edible insects and their potential for integration in contemporary gastronomy. The research was based on data collection by means of a questionnaire survey carried out in 14 countries, involving a total of 7,222 participants. For data processing, analysis of variance (ANOVA) was applied to identify differences between groups, and factor analysis was used to assess the structure of the items on the scale used.

The results indicated greater agreement among participants with the perception that insects are foods associated with exoticism, as well as cultural beliefs, taboos, and resistance to consumption. It was also found that certain sociodemographic characteristics, namely country of origin, age, level of education, and income, significantly influenced perceptions about edible insects ($p < 0.05$). In contrast, sex and place of residence did not show relevant effects. Factor analysis allowed identifying three principal dimensions: F1 – acceptance of insects in gastronomy, F2 – perception of insects as food, and F3 – vision of insects as inappropriate for human consumption (**Figure 1**). [3]

Overall, the study shows that perceptions about edible insects vary between countries and are influenced by different sociodemographic factors, highlighting the importance of cultural and individual aspects in the acceptance of these foods in future diets.

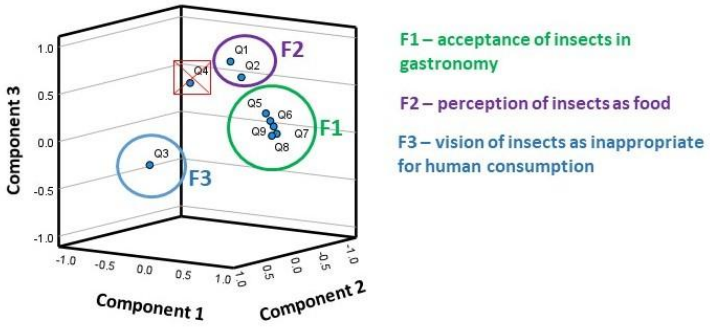


Figure 1 – Factorial structure of the nine items of the scale used to investigate consumer perceptions about edible insects.

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Sensory profile and consumer perception of burgers (conventional and vegetarian)

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Consumers' growing interest in meat alternatives, driven by concerns about health, sustainability, and ethics, has spurred diversification in the food market, with burgers serving as an emblematic product of this transition. We previously analysed the nutritional profile of three types of burgers.[1] We expanded on our previous findings by determining the sensory profile and consumer perception based on a food questionnaire. The samples included a 100% meat burger (made with select Aberdeen Angus beef), a hybrid burger (beef with 25% vegetables, "meat & veggies"), and a vegetarian burger. In the sensory analysis, a global hedonic acceptability test and purchase intention assessment were conducted to evaluate the acceptance of the three distinct burgers. The test was conducted at an external testing facility, in accordance with ISO 11136:2014/Amd 1:2020 and in compliance with the conditions described in ISO 8589:2007/Amd 1:2014 regarding the testing environment. Thirty untrained tasters participated, ranging in age from 23 to 63, of both genders, and who were regular hamburger consumers. The evaluation was conducted using a 9-point hedonic scale, with ratings assigned for the following attributes: appearance, tenderness, juiciness, flavor, overall liking, and purchase intention. The data were statistically analysed using nonparametric tests. Initially, the Friedman test was applied to verify the existence of statistically significant differences among the three hamburgers. When this test indicated relevant differences, the Wilcoxon test was applied to identify specific comparisons between pairs of samples. The confidence level considered was 95%. To complement the analyses, an online questionnaire (n = 240 valid responses) was developed and administered to assess certain assumptions regarding consumers' motivations, perceptions, and eating habits related to different types of burgers. In terms of overall acceptance, the "meat & veggies" burger obtained the highest average score of 6.7 ± 1.2 (93% positive ratings), followed by the Angus burger with 5.9 ± 1.6 (80%), and in third place, the vegetarian burger with an average of 4.4 ± 2.0 (53%) ($p < 0.05$). The three main factors influencing burger choice were found to be the quality of the meat or ingredients (71.8%, 168 individuals), taste (59.8%, 140 individuals), and price (30.3%, 71 individuals). In addition to these three factors, there are others that received some attention, such as being the healthier option (19.2%), market availability (11.5%), following a vegetarian/vegan diet (4.7%), and environmental sustainability (4.7%). The results of the sensory analysis revealed clear differences in how the three burgers were perceived by consumers. The hybrid burger was the most popular, receiving good ratings for flavour, juiciness, and overall acceptance, while the vegetarian burger received lower ratings, mainly due to its less appealing texture and flavour. The consumer questionnaire confirmed a widespread preference for beef burgers, although with a growing openness to hybrid alternatives, and indicated that most consumers maintain more conventional eating habits, valuing product quality and flavour, while remaining open to alternatives with lower meat content. The dominant criteria in purchasing decisions were health and sensory pleasure, while environmental and ethical factors are what lead to change. This study contributes to the discussion on food innovation, health, sustainability and consumer behaviour.

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Mineral Composition and Heavy Metal contamination in three Avocado (*Persea americana* L.) Cultivars: Implications for Food Safety

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Avocado (*Persea americana* L.) is a nutritionally valuable tropical fruit whose mineral composition can vary significantly depending on cultivar and fruit tissue. Thus, given the increasing consumption of avocado and its peel-derived by-products, a comprehensive characterization of its elemental composition, including potential heavy metal contamination, is essential to ensure food safety.

In this context, the present study aimed to evaluate the physicochemical properties and mineral composition of three avocado cultivars (Fuerte, Hass, and Ryan) commercially available in Portugal, considering three tissue fractions: peel-pulp mixture, pulp only, and peel only (Figure 1). The fruit samples were characterized by height, diameter, total soluble solids (TSS), dry matter content, and colorimetric parameters (CIE L*, a*, b*) of both inner and outer tissues. The concentrations of magnesium (Mg), phosphorus (P), sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and lead (Pb) were determined in the peel-pulp mixture, pulp, and peel of each cultivar. Hass exhibited the highest total soluble solids (17 °Brix) and the lowest moisture content (58 %), whereas the Fuerte cultivar showed the highest firmness and moisture content (81 %), reflecting cultivar-dependent differences in fruit maturity and textural characteristics. Potassium was the predominant mineral in all cultivars and tissue fractions, with concentrations ranging from 12,575 mg/kg in Ryan peel to 36,195 mg/kg in Fuerte pulp, followed by calcium and sulfur. Calcium and manganese preferentially accumulated in the peel, whereas potassium was more abundant in the pulp, particularly in the Fuerte cultivar, indicating a tissue-dependent mineral distribution. Magnesium concentrations were below the limit of detection in most samples, except for the Ryan cultivar, where markedly higher concentrations were detected in both the pulp (11,143 mg/kg) and peel (15,044 mg/kg). Lead (Pb) was detected in all cultivars and tissue fractions, with consistently higher concentrations in the peel (26.33 - 49.67 mg/kg) than in the pulp (7.33 - 12.33 mg/kg). Fuerte exhibited the highest Pb concentration in the peel and the lowest in the pulp among the three cultivars. This distribution pattern suggests preferential Pb accumulation in the peel, indicating limited transfer to the edible pulp. Overall, these findings demonstrate significant differences - specific to each variety and tissue - in the physicochemical properties and mineral composition of avocados commercialized in Portugal. The consistent detection of Pb, particularly in the peel, highlights the need for continuous monitoring of heavy metal contamination, especially considering the increasing use of avocado peel in food ingredients and value-added by-products.



Figure 1 – Representative samples of avocado (*Persea americana* L.).

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Dietary Fiber Content in Representative Foods of the First Harmonized Total Diet Study in Portugal

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Background: Dietary fiber is a key component of a healthy diet. The European Food Safety Authority (EFSA) recommends a daily intake of 25 g of dietary fiber for adults. Research has shown that increasing fiber intake can reduce the risk of cardiovascular disease, type 2 diabetes, obesity, and gastrointestinal disorders [(1).]

Accurate analytical data on fiber content in foods reflecting national dietary patterns are critical for nutritional assessment, food composition databases, and public health policy. Total Diet Studies (TDS) have been adopted as a cost-effective approach for assessing population exposure to contaminants and dietary intake of nutrients. The harmonized European TDS methodology, developed within the framework of the TDS Exposure project, enables the comparability of exposure and nutrient intake estimates across countries and within the same country over time [(2)].

Objective: To quantify the dietary fiber content of foods representative of the Portuguese diet within the Portuguese Total Diet Study (TDS), and to characterize its distribution across major food groups.

Methods: The food sampling process included 164 different Total Diet Study (TDS) composite samples for laboratory analysis. Each TDS sample consisted of 12 subsamples and covered approximately 90% of the diets of adults and older adults. Foods were classified into 17 food groups based on their characteristics, following the FOODEX2 classification system. Total dietary fiber was determined on a fresh-weight basis using the AOAC Official Method 985.39 (enzymatic–gravimetric method)[(3)]. Descriptive statistics (mean, standard deviation, and range) were calculated for each food group.

Results: Dietary fiber content varied widely across food groups, ranging from below the limit of quantification (≤0.4 g/100 g) to 15.0 g/100 g fresh weight. Legumes, nuts, oilseeds, and spices showed the highest mean fiber content (8.2 ± 3.5 g/100 g; n = 8), followed by Grains and grain-based products (3.6 ± 2.9 g/100 g; n = 15). Fruit and fruit products (2.0 ± 2.1 g/100 g; n = 14) and Vegetables and vegetable products (2.0 ± 1.1 g/100 g; n = 19) showed intermediate values. Higher fiber values were observed in legumes (15.0 g/100 g) and cereal products (14.5 g/100 g), consistent with Portuguese dietary intake data, which identify these groups as primary contributors of fiber.

Conclusions: Marked differences in dietary fiber content were found across food groups. Legume and Grain products were identified as the richest sources, with fruits and vegetables also making meaningful contributions. These findings, generated within the Portuguese TDS, provide robust, nationally representative data to support food composition databases, nutrient intake assessment, and evidence-based nutritional policy.

Table 1. Dietary fiber content in TDS samples by FoodEx2 food group (g/100 g)

FoodEx2 Level 1 food Groups	n	Mean	Standard deviation	Minimum - Maximum
2- Alcoholic beverages	2	a)	-	-
3- Animal and vegetable fats and oils	2	a)	-	-
4 – Coffee, cocoa, tea and infusions	4	0.40	-	0.40-1.40
5 – Composite dishes	34	1.81	1.19	0.40-5.40
6- Eggs and egg products	1	a)	-	-
7 – Fish, seafood, amphibians, reptiles and invertebrates	25	0.17	0.44	0.40-1.40
9 – Fruit and fruit products	14	1.99	2.11	0.50-9.60
10 – Fruit and vegetable juices and nectars	2	0.40	0.00	0.40-0.40
11 – Grains and grain-based products	21	3.60	2.90	0.80-14.5
12 Legumes, nuts, oilseeds and spices	8	8.20	3.48	3.30-15.0
13- Meat and Meat products	11	a)	-	-
14-Milk and dairy products	6	a)	-	-
15 Products for non-standard diets, food imitators and food supplements	2	3.70	3.54	1.20-6.20
16 – Seasoning, sauces and condiments	5	0.28	0.44	0.40-1.00
17 – Starchy roots or tubers and products thereof, sugar plants	1	1.80	-	1.80-1.80
18 – Sugar confectionery and water-based sweet desserts	3	0.30	0.52	0.40
19 – Vegetables and vegetable products	19	1.97	1.12	0.40-6.50
20 – Water and water-based beverages	4	a)	-	-

a) Logical zero

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Processing-Induced FODMAP Changes in Selected Mediterranean Plant Foods: Current Evidence and Implications for Low-FODMAP Product Development

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The Mediterranean diet has gained substantial popularity over recent decades due to its numerous health benefits. This dietary pattern relies heavily on plant-based foods, many of which are high in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) that can prompt gastrointestinal symptoms in individuals with irritable bowel syndrome (IBS). This paradoxical role of food in IBS – as both a trigger of gut symptomatology and potential therapeutic tool – has directed attention towards the low-FODMAP diet. Although this dietary approach is an evidence-based strategy for managing IBS, its restrictive nature may lead to inadequate energy and nutrient intake and compromise long-term adherence. Therefore, food processing strategies that reduce these fermentable carbohydrates could support the development of low-FODMAP functional ingredients, compatible with Mediterranean dietary patterns.

A structured literature review, supported by a systematic search strategy, was conducted in Web of Science, Scopus, PubMed, and Google Scholar from database inception to 15 May 2026 to assess the impact of domestic and industrial food processing on FODMAP content in selected Mediterranean plant-based foods, namely garlic, onion, bell pepper, tomato, and apple. Original peer-reviewed studies reporting quantitative or semi-quantitative data for raw and/or processed foods were eligible. Data on food matrices, processing conditions, analytical methods, individual FODMAPs, and broader carbohydrate fractions were extracted and synthesized narratively because methodological and compositional heterogeneity precluded meta-analysis. A fit-for-purpose methodological quality assessment was used to contextualize the strength and directness of the evidence.

The database search identified 1,706 records, with 18 additional records retrieved through backward and forward citation tracking; 35 studies met the inclusion criteria. Onion and garlic provided the most robust evidence, particularly for processing-induced changes in their high fructan and fructooligosaccharide contents. By contrast, evidence for bell pepper, tomato, and apple was more limited and was frequently based on free sugars, total or reducing sugars, dietary fiber, or other broad carbohydrate outcomes rather than complete FODMAP profiles.

Water-based approaches, such as boiling, blanching, canning, soaking, and pickling were the most promising strategies. Their effectiveness was primarily attributed to the water-soluble properties of several carbohydrates, which promote leaching into the surrounding aqueous medium. Reductions in the edible fraction were therefore most relevant when the processing liquid was discarded; however, they may reflect carbohydrate transfer rather than true degradation. Nevertheless, these methods may alter product quality by affecting its texture, appearance, flavor, and nutritional composition. Thermal processing and fermentation produced heterogeneous and matrix-dependent changes, resulting from competing mechanisms, including carbohydrate concentration due to water loss, while simultaneously promoting carbohydrate hydrolysis, thermal degradation, microbial consumptions, and monosaccharides accumulation. Consequently, a reduction in total sugars or in one FODMAP fraction may be accompanied by an increase in another symptom-relevant fraction, such as free fructose. The interpretation of processing effects was also limited by differences in analytical specificity, processing conditions, comparator selection, reporting units, and the inconsistent analysis of processing liquids.

Overall, water-based approaches showed consistent potential to reduce soluble FODMAP-related carbohydrates in the edible food fraction, although they often negatively affected the food structure. In contrast, fermentation processes demonstrated extreme changes in FODMAP compounds, with carbohydrate consumption and transformation that led to accumulation of smaller fermentable carbohydrates, while producing a final product with desirable sensory attributes. Nevertheless, current food processing strategies are unable to simultaneously achieve significant FODMAP reduction and preserve the structural integrity of food matrices. Therefore, low-FODMAP product development should prioritize, beyond FODMAP reduction, nutritional and functional preservation, and consumer-acceptable sensory attributes to ensure suitability for the long-term management of IBS.

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Influence of altitude on the physicochemical composition and antioxidant properties of Cobrançosa and Negrinha de Freixo Olives

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Olive fruit composition is influenced by genetic, environmental, and agronomic factors[1]. Among the environmental factors, altitude plays an important role, as it affects temperature, daily thermal amplitude, solar radiation, precipitation, water availability, and soil characteristics. These conditions influence olive tree metabolism, affecting vegetative growth, fruit ripening, and the accumulation of compounds responsible for the nutritional and sensory properties of olives and olive oil. Several studies have shown that olive groves located at higher altitudes tend to produce fruits with higher concentrations of phenolic compounds and greater antioxidant capacity[2,3]. This response is associated with increased abiotic stress caused by lower temperatures, higher ultraviolet radiation, and greater daily thermal amplitude, which stimulate the synthesis of secondary metabolites, particularly phenolic compounds. Information regarding the response of olive cultivars to different edaphoclimatic conditions remains limited. The present study evaluated the effect of altitude on the composition of olives from the Cobrançosa and Negrinha de Freixo cultivars. Two olive groves located in the Mirandela region (northeastern Portugal) at different altitudes were selected (Olive Grove 1: 396 m; Olive Grove 2: 302 m). In each grove, five trees of each cultivar were selected, and fruit samples were collected for the determination of maturity index, moisture and total fat contents, total phenolic content, antioxidant activity (DPPH assay), reducing power, and colour parameters (L, a, b*, C*, and h*). The comparison between olive groves located at 396 m and 302 m above sea level demonstrated that altitude influenced the physical and chemical characteristics of olives from both cultivars. For the Cobrançosa cultivar, olives from the grove located at 396 m exhibited a higher maturity index (3.3) than those from the grove at 302 m (2.73). Moisture (59.5%) and fat contents (8.4%) were similar between the two altitudes. However, olives produced at the higher altitude showed greater antioxidant activity (DPPH inhibition = 38.7%), higher total phenolic content (84.4 mg Gallic acid equivalents g^{-1} extract), and greater reducing power (134.0 mg Trolox equivalents g^{-1} extract) than those produced at the lower altitude (33.2%, 68.1 mg Gallic acid equivalents g^{-1} extract, and 117.8 mg Trolox equivalents g^{-1} extract, respectively). Regarding colour, olives from the higher-altitude grove exhibited lower lightness (L* = 39.7) and lower yellowness (b* = 9.7), indicating visually darker fruits. For the Negrinha de Freixo cultivar, olives produced at 396 m also exhibited a higher maturity index (3.0 vs. 2.4). Moisture content was lower at the higher altitude (63.1% vs. 66.1%), as was fat content (8.2% vs. 10.8%). In contrast, antioxidant activity (22.3% vs. 15.1%), total phenolic content (46.8 vs. 32.5 mg Gallic acid equivalents g^{-1} extract), and reducing power (78.6 vs. 51.6 mg Trolox equivalents g^{-1} extract) were significantly higher in olives produced at 396 m. Regarding colour, olives from the higher-altitude grove exhibited lower lightness (L* = 44.2 vs. 48.6) and lower b* values (12.0 vs. 17.6), indicating darker fruits. Comparing the two cultivars, Cobrançosa exhibited higher total phenolic content, antioxidant activity, and reducing power than Negrinha de Freixo at both altitudes, indicating greater intrinsic antioxidant potential. Conversely, Negrinha de Freixo olives showed higher moisture content and, at the lower altitude, higher fat content. Principal component analysis (PCA) supported these findings. Figure 1A showed a partial separation of the samples according to altitude, with the first two principal components explaining 81.5% of the total variance (PC1 = 49.8%; PC2 = 31.7%). Olives from 396 m were associated with higher total phenolic content, antioxidant activity (DPPH inhibition), reducing power, and maturity index, whereas those from 302 m were associated with higher moisture and fat contents. Figure 1B revealed a partial separation according to cultivar and olive grove, with Cobrançosa samples clustering with antioxidant-related variables, while Negrinha de Freixo samples were more closely associated with moisture and fat content. In both PCAs, the overlap between groups indicates that altitude, cultivar, and olive grove only partially explain the observed variability. Overall, these results suggest that higher-altitude environments favour the accumulation of phenolic compounds and antioxidant metabolites, while cultivar characteristics contribute additional variation in the physicochemical and bioactive composition of the olives.

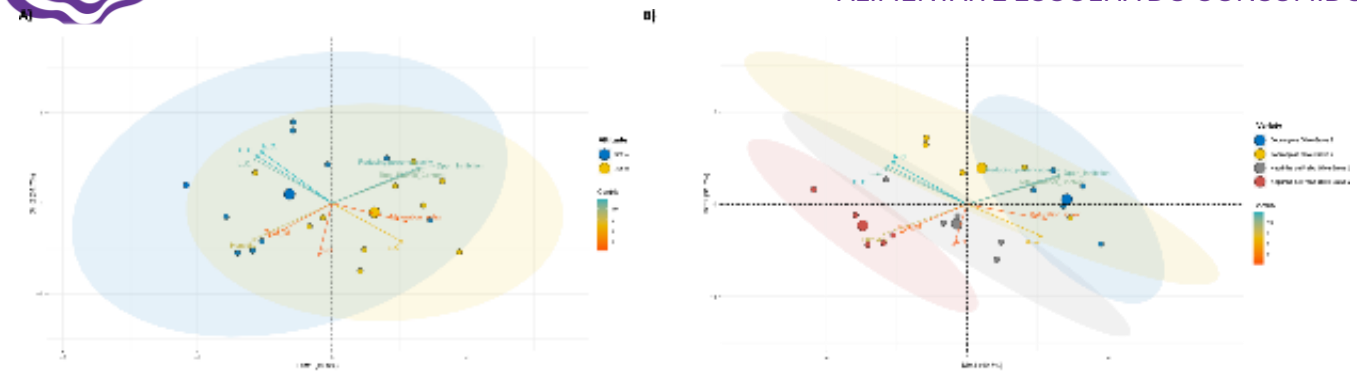


Figure 1. Principal component analysis (PCA) biplots showing the distribution of olive samples according to (A) altitude (302 and 396 m) and (B) cultivar (Cobrançosa and Negrinha de Freixo) and olive grove (Olive Grove 1 and Olive Grove 2), based on physicochemical, colour, and bioactive parameters.

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Science on Social Media: Strategies for Communicating Food Safety to the General Public

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Scientific research generates knowledge, but its societal value depends on how effectively it is communicated beyond academic audiences. Although researchers are trained in experimental design, data analysis, and scientific publication, they often receive limited training in translating their findings for non-specialist audiences, creating a gap between scientific outputs and public understanding. Social media platforms play a central role in shaping the public's access to scientific information, offering new opportunities for dissemination while also being platforms where misinformation can spread rapidly.

In this context, accurate, accessible, and evidence-based communication by scientists is increasingly important. This work presents a case study based on @ritacozinhasegura, an Instagram page dedicated to communicating food safety and food science to the general public, managed by a food safety specialist and PhD student in food chemistry and nutrition. Between January and July 2026, the page reached 1,8 million accounts and generated 4,8 million views on Instagram, an increase of 180,7% compared to the previous period. Content generated 194,6 thousand interactions, an increase of 434,1%, almost entirely from organic reach, currently sustaining a community of over 15,5 thousand followers. These results confirm a genuine public interest in science, provided it is translated into meaningful narratives relevant to diverse audiences, rather than simplified content alone.

Key approaches applied include storytelling structures, visual communication, humanization of the researcher and the scientific process, and diversification of content formats, including short form videos, carousel posts, and stories, each shown to sustain interaction and expand reach differently. Practical strategies discussed include myth debunking, evidence-based food safety tips, and collaborations with brands and media outlets, always maintaining scientific rigor and credibility.

This case study demonstrates that improving science communication does not require transforming researchers into professional communicators, but rather integrating simple, intentional communication practices into dissemination, significantly enhancing the outreach, understanding, and societal relevance of scientific work, and supporting food literacy and informed consumer choices.

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Designing a Low-Sugar Fruit–Botanical Beverage through Chemical, Cellular and Translational Evidence

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Fruits and wild plants are important sources of phenolic compounds with antioxidant, anti-inflammatory and metabolic regulatory properties, supporting their incorporation into functional foods and nutraceutical formulations [1–3]. This study applied an integrated approach that combined beverage formulation, physicochemical characterisation, phenolic profiling, cellular bioactivity, and human biomarker evidence to evaluate sweet cherry–blueberry beverages enriched with *Pterospartum tridentatum* ("carqueja") infusion.

Six beverage prototypes were developed using Prime-Giant sweet cherry juice, blueberry and carqueja. Soluble solids, acidity and CIELAB colour parameters were determined to evaluate formulation quality and stability. Cytocompatibility was assessed in NHDF fibroblasts and HepG2 hepatocytes using metabolic activity and lactate dehydrogenase-release assays. Anti-inflammatory activity was evaluated in lipopolysaccharide-stimulated RAW 264.7 macrophages by measuring nitric oxide production under viability-controlled conditions. The phenolic profiles of Prime-Giant cherries, Legacy blueberries and carqueja infusion were compared. Human relevance was further investigated using data from a 42-day pilot intervention in which participants consumed 280 g of Prime-Giant cherries daily.

The formulation process produced beverages with approximately 30% lower soluble solids content, reaching nearly 7 °Brix while maintaining stable acidity and relatively uniform colour. Formulation 3C provided the most favourable balance between reduced sugar content, physicochemical stability and visual consistency. Carqueja produced the strongest reduction in nitric oxide production; however, this effect was accompanied by increased cytotoxicity at elevated concentrations. Conversely, the fruit-based beverage induced a milder, dose-dependent anti-inflammatory response while maintaining greater cell viability, indicating a broader cytocompatibility range. Phenolic profiling demonstrated complementary chemical compositions: carqueja was mainly characterised by genistein and quercetin derivatives, Legacy blueberry contained anthocyanins and flavonols, and Prime-Giant cherry was dominated by non-coloured phenolics, particularly caffeoylquinic acid derivatives. Human intervention also revealed significant changes in selected biomarkers related to glycaemic regulation, inflammation, oxidative status, and liver function.

These findings identify formulation 3C as a promising low-sugar fruit–botanical beverage and demonstrate the importance of integrating chemical composition, product quality, cellular safety and biological activity during functional beverage development.

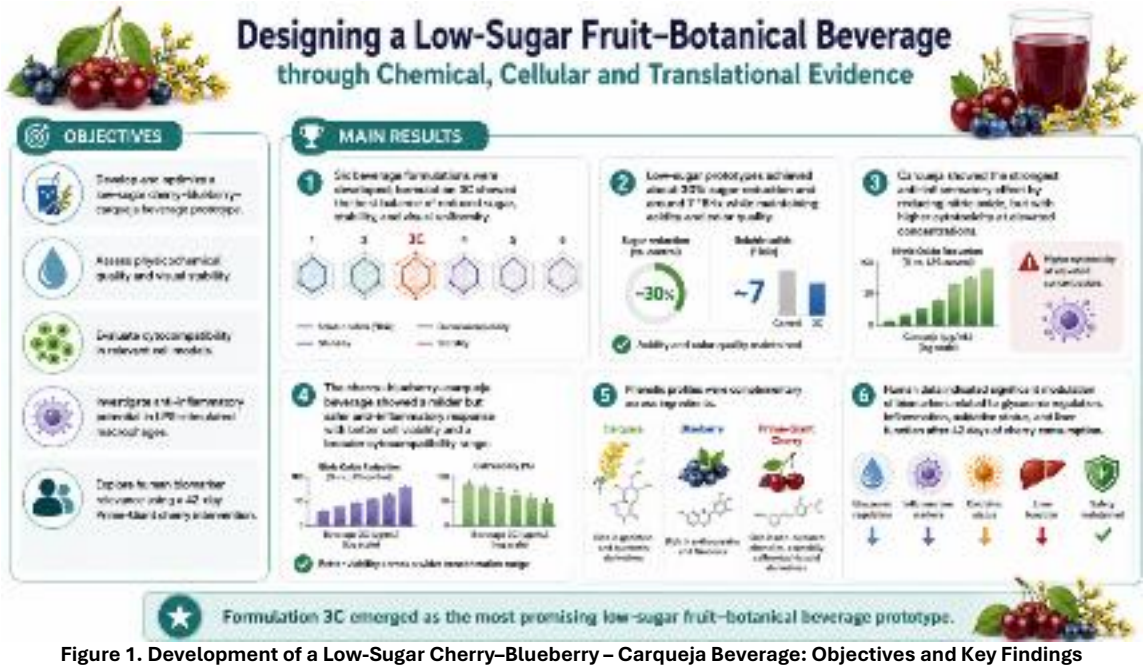


Figure 1. Development of a Low-Sugar Cherry–Blueberry – Carqueja Beverage: Objectives and Key Findings

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Medicinal Plants *Rhus coriaria* and *Cistus ladanifer*: Bioactive Composition and Biological Potential

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Natural products derived from medicinal plants represent an important source of bioactive compounds with potential applications in pharmaceutical and nutraceutical fields. This study aimed to evaluate the phytochemical composition and biological potential of hydroethanolic extracts (HEs) and infusions (INFs) prepared from *Cistus ladanifer* leaves and *Rhus coriaria* fruits. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively. Antioxidant activity was evaluated through DPPH•, nitric oxide (NO•), and superoxide anion (O_2•−) scavenging assays. The inhibitory activity against α-glucosidase, as well as cytotoxic and anti-inflammatory effects, was also investigated using different in vitro models.

The results showed that the extraction method and plant species markedly influenced the phytochemical composition and biological activities of the extracts. The highest TPC was observed for the HE of *R. coriaria* fruits (537.1 ± 8.62 mg GAE/g dry extract), followed by the HE of *C. ladanifer* leaves (514.4 ± 3.08 mg GAE/g dry extract). Regarding TFC, the HE of *C. ladanifer* leaves presented the highest value (43.3 ± 1.77 mg QE/g dry extract), while the corresponding infusion showed 31.6 ± 3.67 mg QE/g dry extract.

The extracts exhibited relevant antioxidant activity in the three assays evaluated. The HE of *R. coriaria* fruits showed the lowest IC_{50} against DPPH• (5.66 ± 0.58 µg/mL), a value close to that of the positive control, ascorbic acid (5.05 ± 0.21 µg/mL). Against NO•, the INF of *C. ladanifer* leaves presented the lowest IC_{50} (29.81 ± 0.62 µg/mL), also close to the positive control (29.66 ± 0.92 µg/mL), whereas the HE of *C. ladanifer* showed the lowest IC_{50} against superoxide anion (31.50 ± 0.38 µg/mL).

All extracts also exhibited α-glucosidase inhibitory activity, with the HE of *R. coriaria* fruits showing the lowest IC_{50} (2.91 ± 0.10 µg/mL), considerably lower than that of the positive control, acarbose (470.2 ± 2.08 µg/mL). In the cell viability assays, an increase in cytotoxicity was generally observed at concentrations equal to or higher than 200 µg/mL, with differences depending on the extract and cell line. The HE of *C. ladanifer* showed the most pronounced effect in Caco-2 cells, while the INF of the same species exhibited greater cytotoxicity in NHDF cells. In RAW 264.7 macrophages, the extracts generally maintained cell viability above 90% within the tested range of 25–200 µg/mL.

Regarding anti-inflammatory activity, the INF of *C. ladanifer* leaves showed the highest potential, producing the greatest reduction in NO production by LPS-stimulated RAW 264.7 macrophages, with NO levels below 50% at extract concentrations of 400 µg/mL or higher.

Overall, the results demonstrate that *C. ladanifer* and *R. coriaria* contain relevant levels of phenolic and flavonoid compounds and exhibit a broad range of biological activities, particularly antioxidant, α-glucosidase inhibitory and anti-inflammatory effects. The observed differences between extracts highlight the influence of plant species and extraction method on their phytochemical composition and biological properties, supporting further investigation of these species as potential sources of natural bioactive compounds.

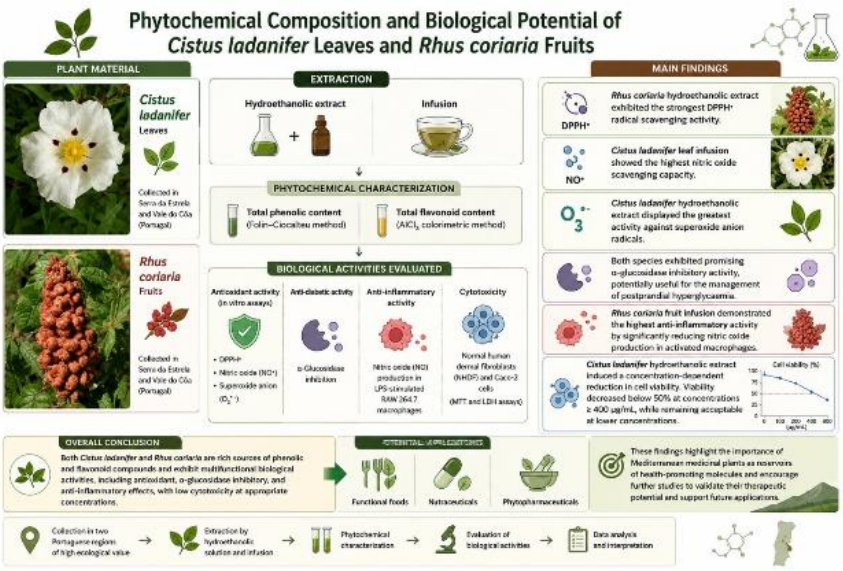


Figure 1 - Schematic overview of the study design, phytochemical characterisation, biological activities, and potential applications of *Cistus ladanifer* and *Rhus coriaria* extracts and infusions.

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Monitoring and Reduction of Salt Content in School Meals

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School meals represent an important opportunity to promote the development of healthy eating habits from an early age and should ensure the provision of nutritionally balanced meals. Evidence suggests that balanced school meals are associated with improved students' concentration and cognitive performance in the classroom.

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend a maximum daily intake of 5 g of salt, equivalent to 2 g of sodium, as a strategy for the prevention of hypertension (HTA) [(1)].

Excessive salt intake represents one of the major public health challenges in Portugal, as salt consumption almost doubles the WHO-recommended maximum level of intake, highlighting the urgent need to implement measures aimed at reducing salt consumption. High sodium consumption during childhood is associated with an increased risk of elevated blood pressure and may contribute to the development of cardiovascular disease later in life. Therefore, reducing salt intake from an early age is a public health priority [(2)].

The "Lunch less salt = better health" program is implemented in the municipality of São João da Madeira and involves all 15 public schools in the local school network. Its main objective is to monitor and reduce the salt content of school meals, contributing to the promotion of healthier eating habits among children and adolescents.

In this edition, the project has been expanded to include the monitoring of the salt content not only of the soup but also of the main course served in school canteens. Monitoring is carried out by collecting meal samples without prior notice at three points during the school year, enabling assessment of progress over time and evaluation of the impact of the measures implemented.

In parallel, the project aims to strengthen its educational component by providing training for the kitchen staff in schools, in collaboration with the Local Health Unit and the school meal service provider. This initiative promotes the adoption of good cooking practices and the gradual reduction of added salt, in line with public health recommendations.

The purpose of this work was to assess the salt content of school meals collected from 15 participating schools at three time points, followed by an intervention phase, to evaluate their contribution to children's dietary salt intake.

The sodium contents of soup and main course samples (n = 92) were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES) after an acid digestion. The corresponding salt content was calculated based on sodium concentration using the conversion factor: salt = sodium × 2.5.

Salt content ranged from 0.3 to 3.5 g/meal in the first monitoring period, from 0.4 to 4.6 g/meal during the second, and from 0.2 to 1.7 g/meal in the third. Overall, salt content decreased by 36% to 51% over the course of the program. The mean salt content of the school meals was consistent with the recommended maximum daily salt intake (<5 g NaCl/day).

Continuous monitoring, staff training and technical support, will make it possible to assess changes in the salt content of school meals served and evaluate the impact of the measures implemented. This project highlights the importance of combining a regular monitoring with capacity-building initiatives for school catering staff, encouraging the adoption of best practices in meal preparation and supporting the progressive reduction of added salt. Through collaboration between schools, the Local Health Unit, and the school meal service provider, the project aims to improve the nutritional quality of school meals, promote healthier dietary habits among students, and contribute to the health and well-being of the school population.

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Healthy Food Access and Availability in a Higher Education Institution: Perceptions of the Academic Community

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Introduction: The food environment in higher education institutions may influence the food choices of the academic community and represents an important context for the adoption of healthy eating habits. The characteristics of the food supply available within these settings, including the variety, availability, attractiveness, and perceived affordability of food options, may facilitate or hinder healthy food choices[1,2]. Understanding how members of the academic community perceive the food environment is therefore important for identifying barriers associated with the selection of healthy foods and for recognizing areas where improvements may be needed. Assessing these perceptions can provide relevant information about the extent to which the food environment is perceived as supportive of healthy eating within a higher education setting[1,2].

Objective: To assess the perceptions of the academic community of a higher education institution regarding access to and availability of healthy foods, as well as the factors influencing the choice of healthy food options in the institution's food outlets. The study also aimed to examine whether these perceptions differed according to participants' gender, age group, or institutional affiliation.

Materials and Methods: An observational, cross-sectional, quantitative study was conducted using an online questionnaire administered to members of the academic community of a higher education institution. A total of 215 individuals participated in the study. The questionnaire addressed participants' perceptions of the food supply, including the availability and accessibility of healthy food options, ease of identifying healthy foods, satisfaction with the available food supply, and factors that may influence the choice of healthy options. Perceptions regarding the affordability of food options were also assessed. Data were analysed using descriptive and inferential statistics in order to characterize participants' perceptions and examine differences according to gender, age group, and institutional affiliation. Statistical significance was set at $p<0.05$.

Results: Most participants perceived the food supply available within the institution as only moderately supportive of healthy food choices, suggesting that the existing offer was not consistently perceived as facilitating the adoption of healthier eating habits. The main barriers identified were the limited variety of healthy food options, reported by 48.8% of participants, and the reduced attractiveness of these options, reported by 26.5%. These findings indicate that both the range and attractiveness of healthy food choices were relevant aspects of participants' perceptions of the institutional food environment. Statistically significant differences were observed according to gender. Female participants reported greater ease in identifying healthy foods, higher satisfaction with the food supply, and a more favourable perception of the affordability of healthy food options ($p<0.05$). However, no statistically significant differences were found between age groups or according to institutional affiliation for most of the variables analysed.

Conclusions: The findings highlight the need to improve the food environment of the higher education institution studied, particularly by increasing the variety, attractiveness, and availability of healthy food options. The perceptions reported by participants point to limited variety and reduced attractiveness as relevant barriers to healthy food choices within the institutional setting. Strategies aimed at promoting healthy eating and improving the characteristics of the available food supply may contribute to facilitating more balanced food choices among members of the academic community. These strategies should consider the perceptions identified in this study and focus on making healthy food options more varied, attractive, and accessible within the institution.

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Promoting Food Literacy Through Nutrition Label Education: A Quasi-Experimental Study in Adults with Mild Neurodevelopmental Disorders

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Introduction: Food literacy is an important component of health literacy, contributing to individuals' ability to understand and use food- and nutrition-related information when making dietary decisions[1]. Food labels can serve as an important tool for supporting informed food choices by providing information that enables consumers to better understand the nutritional characteristics of food products[2]. However, interpreting nutritional information and using it effectively in everyday food choices may present challenges, particularly among individuals with specific needs, such as people with mild neurodevelopmental disorders. In this context, nutritional education and accessible educational strategies may contribute to improving knowledge and supporting greater autonomy in food-related decision-making. Promoting the ability to understand and interpret food labels is therefore relevant to the development of food literacy and to the empowerment of consumers in their food choices[1].

Objective: To promote food literacy through nutritional education, with a particular focus on understanding and interpreting food labels, and to assess changes in participants' knowledge and reported behaviours related to food label reading following an educational intervention among adults with mild neurodevelopmental disorders.

Materials and Methods: A quasi-experimental study was conducted at the Centro de Integração e Reabilitação de Tomar (CIRE). The sample comprised 11 adults with mild neurodevelopmental disorders, including 4 females and 7 males, with educational levels ranging from primary to secondary education. Considering the characteristics of the sample, six practical educational sessions were conducted. The intervention focused on the interpretation and practical understanding of food labels and involved six food products: yogurts, Nestum, potato chips, juice, ham, and Maria biscuits. A measuring cup and a scale were also used during the sessions. Participants were involved in weighing and measuring the food products, allowing them to understand the nutritional information provided per 100 grams and per portion. The intervention therefore combined educational content with practical activities designed to facilitate the understanding and use of nutritional information. Knowledge and reported behaviours related to food labels were assessed before and after the intervention (T0 and T1), including participants' knowledge of nutrition labels and Nutri-Score, their habit of reading food labels, their perception of the importance of reading labels, and the reported frequency with which they went shopping and analysed food labels.

Results: Following the intervention, 63.6% of participants showed an improvement in knowledge. Statistically significant differences were observed in responses to the questions "Do you know what a nutrition label is?" and "Do you know what Nutri-Score is?". These findings indicate an improvement in participants' knowledge regarding food labelling and Nutri-Score following the educational intervention. However, no statistically significant changes were observed between T0 and T1 regarding the habit of reading food labels or the perceived importance of this practice. Similarly, the frequency with which participants reported going shopping and analysing food labels remained stable, with no statistically significant differences observed between the two assessment moments. Thus, although the intervention was associated with improvements in knowledge, these changes were not accompanied by statistically significant changes in the reported behaviours related to food label use.

Conclusions: This study demonstrates that an inclusive educational intervention focused on food labels can contribute to improvements in food-related knowledge among adults with mild neurodevelopmental disorders. The observed increase in knowledge regarding nutrition labels and Nutri-Score highlights the potential of practical nutritional education strategies to support food literacy and informed consumer food choices in populations with specific needs. However, the absence of statistically significant changes in reported label-reading habits, perceived importance of label reading, and the frequency of analysing labels suggests that improvements in knowledge did not translate into measurable behavioural changes over the period assessed. These findings highlight the importance of sustained educational approaches, continuous reinforcement, and integration into everyday contexts to facilitate the practical application of acquired knowledge. Strengthening food literacy through accessible and inclusive strategies may contribute to greater autonomy and support consumers in making more informed food choices.

Acknowledgments: The authors would like to thank the Centro de Integração e Reabilitação de Tomar (CIRE) for their collaboration and support throughout this research.
Funding: No funding was received for this work.

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XVIII **Encontro** de **Química** dos **Alimentos**

Coimbra | 14 - 16 de outubro'26

PATROCÍNIOS

Food safety, quality and authenticity - Agilent solutions for food and beverage characterization and quality control assays

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Ensuring food safety and quality is an increasing challenge. Whether in the analysis of contaminants or the authenticity of label claims, Agilent seeks to find analytical solutions for customers in various applications with excellent equipment with the forefront of technology.

Food safety testing1 is critical in today's regulated environment. Much of the food we eat and enjoy today is provided through complex global food production, processing and distribution systems. Analytical testing at all stages of the supply chain is essential to ensure food safety and quality. Laboratories face challenges in maximizing efficiency and productivity, while also being able to determine contaminants at ever-lower concentration levels while meeting evolving testing regulations. Agilent supplies best-in-class chromatography equipment with mass spectrometry detectors (GC-MS and LC-MS) with unique performances for identifying and quantifying diverse contaminants such as pesticides, mycotoxins, PFAS, PAH's and veterinary drug residues in various food matrices, as well as microplastics presence in food chain.

Accidental adulteration or intentional food fraud affects “certified” quality products such as specialty cheeses, premium wines, tea from endemic plants and regional olive oils, as well as staple foods such as rice and milk. Analytical testing is essential to ensure Food Authenticity2 and consumer safety and protect industry brands. Agilent has the analytical equipment to help you meet all your food authenticity needs.

The food industry must also adapt to respond to consumers who demand to know more about the products they are buying, and the impact this food products have on their health and nutrition3. The inclusion of food additives (sugars, salts, fats and dye), the nutritional value of foods and diathetic supplements and the metabolic impacts on our health are factors of concern to consumers. Agilent, with its wide range of high-sensitivity analytical equipment, contributes to the development of ever faster, more accurate and sensitive methodologies that allow the complete characterization of the nutritional impact of food on human health.

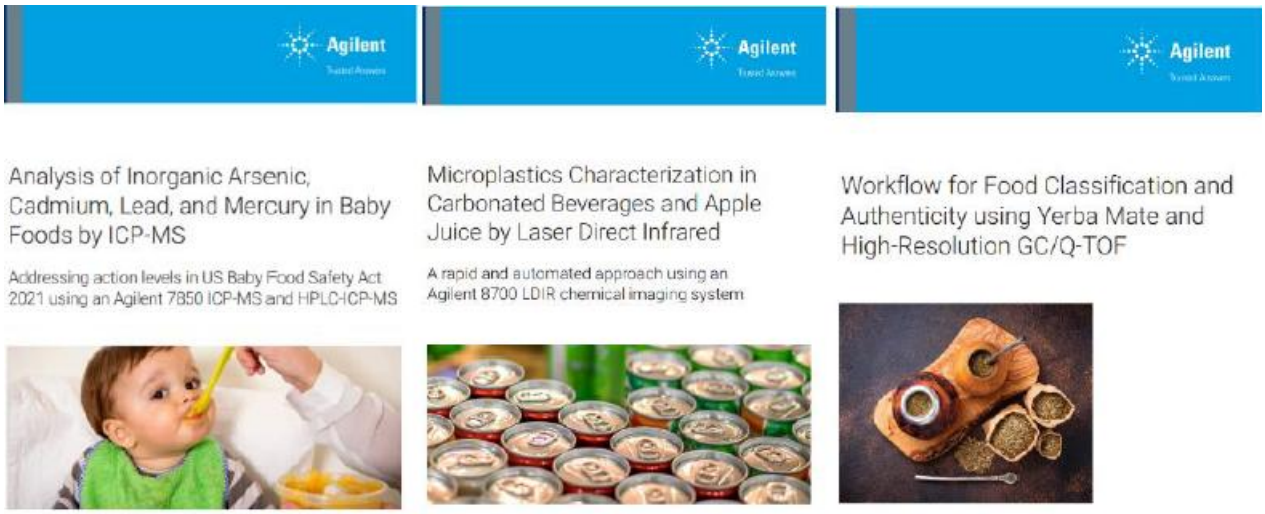


Figure 1: Agilent Application Notes on food safety, quality and authenticity assays.

References:

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Insects as an Alternative Protein Source: N/Protein Determination Analytical Methods and Challenges

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With the global population expected to reach nearly 10 billion by 2050, the food industry is actively investigating sustainable alternative protein sources. Edible insects and their derivatives have emerged as a highly promising solution due to their rich nutritional profile, particularly high crude protein content (ranging from 40% to 60%). However, their industrial scale-up faces key hurdles, including consumer perception, inconsistent documentation of nutrient profiles, and, critically, the lack of official, standardized, and validated analytical methods for determining nitrogen and crude protein content in insect-based products.

This study evaluates and compares the two primary reference techniques for Total Nitrogen and Crude Protein determination in insect meals: the Dumas combustion method (using the VELP NDA 702) and the Kjeldahl wet digestion method (using the VELP UDK 169). Method development protocols for both approaches are outlined, addressing crucial pre-analytical and operational steps such as sample drying, homogenization, sample size optimization, reagent selection, and combustion/digestion conditions.

Experimental testing on various insect flour samples demonstrated excellent agreement between the two techniques. The Dumas method yielded average nitrogen values of 9.226% (±0.018, RSD 0.190%), 9.328% (±0.062, RSD 0.669%), and 8.458% (±0.066, RSD 0.779%) across three representative insect meal types, corresponding to protein contents of 57.660%, 58.302%, and 52.865%, respectively (using the default N x 6.25 conversion factor). Comparative analysis using the Kjeldahl method on the same samples provided matching results within statistical limits: 9.194% N (57.463% protein), 9.326% N (58.290% protein), and 8.418% N (52.614% protein), with low Relative Standard Deviations across all trials (RSD < 0.8%).

Both analytical frameworks prove robust, reliable, and suitable for key industry applications, including raw material quality control, final product verification, and nutritional labeling. Selecting between these methods depends on specific laboratory requirements: the Dumas method offers rapid turnaround time, high sample throughput, automation, and a chemical-free operational workflow, whereas the Kjeldahl method remains a highly versatile, cost-effective standard for low-to-medium sample volumes. These findings provide clear guidelines for food testing laboratories navigating the emerging regulatory landscape and regulatory standards (e.g., EU Novel Food Regulation 2023/5 and US FDA frameworks) for edible insect processing.

Ainda precisamos do Kjeldahl? Uma nova abordagem à determinação de azoto e proteína por combustão Dumas

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A determinação de azoto e proteína é uma análise habitual nos laboratórios da área alimentar, sendo o método de Kjeldahl uma das metodologias mais conhecidas e utilizadas. No entanto, existem atualmente alternativas que permitem realizar esta análise de forma mais rápida, simples e automatizada.

Nesta apresentação será feita uma breve introdução ao método de combustão Dumas, comparando-o com o método de Kjeldahl e destacando as suas principais vantagens para o trabalho de rotina.

Será apresentado o rapid N exceed PLUS, da Elementar, uma solução para a determinação de azoto e proteína que permite obter resultados em poucos minutos, com elevada capacidade de automatização e reduzida intervenção do operador. A ausência de etapas de digestão e a eliminação da utilização de reagentes agressivos são também vantagens relevantes desta abordagem.

Serão ainda apresentados alguns exemplos da aplicação do equipamento na análise de alimentos, mostrando como a combustão Dumas pode contribuir para tornar a determinação de azoto e proteína mais rápida e simples.

Leco

One Analyzer, Multiple Solutions: From MOSH, MOAH, Pesticides, and Beyond

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LECO EATC, Berlin, Germany, 2 LECO Corporation, St. Joseph, MI, USA

Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) is nowadays considered a robust analytical technique for the detection and quantification of various target and non-target compounds in food matrices.

This technique has already proven to be highly effective for the qualitative analysis of Mineral Oil Hydrocarbons (MOH), revealing markers of contamination (e.g., hopanes, dibenzothiophenes, diisopropylnaphthalenes, POSH, PAO) as well as natural interferences (e.g., terpenoids).Recently, LECO also introduced a more advanced solution, including a GC×GC-FID channel within the same hardware setup, enhancing the accuracy of quantification for these fractions while using GC×GC-MS data to support quantitative results.

Due to the intrinsic characteristics of TOFMS, such as the acquisition of full-mass range data with superior sensitivity, similar or slightly adapted methods can also be used to detect and quantify other compound classes, both as targets but also as unknowns, with simultaneous high quality non-target data collection. This adaptability addresses additional complex analytical challenges. For instance, we have applied nearly the same method to detect and quantify pesticide residues in food commodities and furans/methyl furans in coffee and coffee-substitute samples, ensuring compliance with regulatory standards, whilst obtaining significant insights into the presence of other residues and sample characterising analytes such as aroma active species.

Examples of the combined analytical methods will be presented, highlighting the synergistic effect of using one technology to address multiple analytical questions.

Bruker

Decoding Wine: Advanced Analytical Solutions for Quality, Authenticity and Innovation

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The wine industry faces increasing demands for quality assurance, product authenticity, regulatory compliance, and production optimization. Advanced analytical technologies now provide unprecedented insights into wine composition, enabling producers to better understand, protect, and enhance their products throughout the entire production chain.

This presentation will showcase Bruker's comprehensive portfolio of analytical solutions for the wine sector, covering applications ranging from cork screening and taint prevention to the determination of contaminants and pesticide residues. The session will also explore the impact of different storage and closure conditions on wine and sparkling wine quality, enabling objective comparisons between alternative cork and packaging solutions.

Additional topics will include wine fingerprinting and profiling, phenolic compound characterization, authenticity verification, and the use of analytical data to differentiate wines according to origin, variety, and production practices. Through real-world case studies, attendees will discover how modern analytical technologies can support quality control, product development, brand protection, and consumer confidence in an increasingly competitive global market.

Join us to explore how cutting-edge analytical science is helping the wine industry transform data into actionable insights, from vineyard to bottle.

Upcycling of food side streams: the benefits of twin-screw granulation of apple pomace

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Sustainable food production and circularity are increasingly important for reducing waste generated by the food industry. Apple pomace, the major by-product of apple juice production, is rich in nutrients but its poor techno-functional properties and low solubility limit its further use as a food ingredient. Extrusion technology provides a continuous and versatile approach for modifying these properties through the simultaneous application of thermal and mechanical energy.

This work investigated twin-screw granulation as a strategy to functionalize apple pomace. Extrusion trials were performed using an 11 mm co-rotating Thermo Scientific™ Process 11 Twin-Screw Extruder equipped with a granulation kit and open discharge. Apple pomace was processed at different throughputs (0.2, 0.4 and 0.6 kg/h), with 23 wt% added water, and screw speeds of 400 or 600 rpm. The resulting material was characterized by oscillatory rheology and scanning electron microscopy to evaluate its gelation behavior and particle structure.

Twin-screw wet granulation improved the techno-functional properties of apple pomace, producing a porous powder with high water-binding capacity and the ability to form a gel/paste immediately after the addition of water. Rheological measurements showed that all granulated samples formed a gel/paste when mixed with water, with storage moduli higher than the corresponding loss moduli. However, gel strength and gelation kinetics were influenced by throughput and consequently by the residence time inside the extruder.

Particle morphology was also affected by throughput. Scanning electron microscopy showed that lower throughput resulted in looser and more porous particles, whereas higher throughput produced more compact structures. These differences in porosity may contribute to the different swelling and gelation behaviors observed.

Screw speed was another relevant processing parameter. Increasing the screw speed from 400 to 600 rpm significantly decreased the storage and loss moduli and consequently the gelling/swelling properties of the granulated apple pomace. According to the application study, higher thermo-mechanical treatment may cause degradation of polysaccharides involved in water binding and gel formation.

Overall, the results demonstrate that twin-screw granulation is a promising technology for the functionalization of apple pomace from fruit juice production. By varying processing conditions, particularly throughput and screw speed, different gelling and swelling properties can be obtained. At the same time, excessive thermo-mechanical treatment should be avoided, as it can lead to polysaccharide degradation and reduced gelling properties. Twin-screw granulation therefore represents a potential approach for the upcycling of food industry waste streams.

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PFAS in Food-Contact Packaging: LC-MS/MS Strategies to Address Regulation (EU) 2025/40

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Per- and polyfluoroalkyl substances (PFAS) have been used to provide oil, grease and moisture resistance to food-contact packaging. Their chemical diversity, environmental persistence and possible presence in coatings, inks, adhesives and polymers create a demanding analytical challenge.

From 12 August 2026, Regulation (EU) 2025/40 establishes three concentration limits for PFAS in food-contact packaging: 25 µg/kg for any individual PFAS measured by targeted analysis, 250 µg/kg for the sum of PFAS measured by targeted analysis, where applicable following prior degradation of precursors, and 50 mg/kg for PFAS including polymeric PFAS. These thresholds require complementary analytical information rather than a single measurement principle.

This presentation discusses how liquid chromatography-tandem mass spectrometry (LC-MS/MS) can support the first two criteria and contribute to a broader compliance strategy. Because PFAS can originate from laboratory materials and LC systems, procedural blanks, reagent quality, carryover assessment and discrimination of system-derived background are integral to reliable quantification.

Combining targeted LC-MS/MS with precursor and fluorine information, where justified, provides a scientifically defensible strategy for evaluating PFAS in food-contact packaging under Regulation (EU) 2025/40.

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T Beyond Sensitivity: The Combined Impact of Advanced Sample Preparation and Triple Quadrupole ICP-MS for Trace Element Analysis in Baby Food

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The determination of toxic and essential elements in baby food represents a demanding analytical challenge due to the wide variety of sample matrices, complex compositions and increasingly stringent regulatory requirements. Reliable results at trace and ultra-trace levels depend not only on ICP-MS sensitivity, but also on the quality, reproducibility and robustness of sample preparation.

This work presents an integrated analytical workflow for the determination of trace and nutritional elements in a wide range of baby food matrices, combining advanced sample preparation technologies from Milestone with Thermo Scientific triple quadrupole ICP-MS. Particular emphasis is placed on automation, reproducibility and interference control as key factors for achieving reliable results in routine laboratories.

Different baby food matrices, including dry powders, cereals, biscuits, dairy products, fruit and vegetable preparations, meat and fish, were processed using microwave-assisted acid digestion with the Milestone UltraWAVE SRC Technology, allowing a common digestion strategy to be applied to highly diverse matrices. The UltraWAVE Single Reaction Chamber technology enables efficient digestion of relatively high sample masses using controlled temperature and pressure conditions. Automated reagent addition using the Milestone EasyFILL acid dispenser further improves reproducibility while reducing operator intervention.

The presentation will additionally discuss the role of controlled acid preparation and acid purification/distillation as important elements in minimizing contamination and improving blank control when working at very low concentration levels.

The resulting solutions were analysed using an iCAP MTX ICP-MS, operated in both single-quadrupole KED and triple-quadrupole oxygen reaction modes. The use of TQ-O_2 provides an additional level [1] of control over spectral interferences compared with conventional single-quadrupole ICP-MS, enabling the effective removal of problematic polyatomic and isobaric interferences while maintaining the sensitivity required for regulated elements.

The workflow [2] achieved excellent linearity and method performance, with method quantification limits for the critical toxic elements As, Cd, Hg and Pb at least two-fold below the nominal limits referenced in the bibliography. Certified reference materials demonstrated recoveries between approximately 90 and 120% for these critical elements, confirming the accuracy and reproducibility of the complete workflow.

Conclusion:

The results demonstrate that reliable trace-element analysis begins with controlled and reproducible sample preparation and is subsequently strengthened by the selectivity of triple-quadrupole ICP-MS. The combination of Milestone automated digestion technology and advanced ICP-MS interference control provides a robust approach for laboratories facing increasingly demanding detection limits, complex food matrices and high-throughput routine analysis.

Acknowledgements: We would like to thank *Laurent Naëls EMEA Customer Solution Center, Thermo Fisher Scientific France for the data provided*

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From mixing to mouthfeel: Complementary rheological and tribological tools for plant-based food development

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Developing plant-based foods requires an understanding of how ingredient composition and processing conditions influence structure, flow and oral lubrication. Protein-rich ingredients and starches contribute to these properties, making their characterization relevant to both formulation and process development.

This contribution presents a complementary instrumental approach, illustrated through selected Anton Paar and Brabender application studies, linking laboratory-scale processing with rheological and tribological characterization. Mixing tests and torque-based measurements provide information on the consistency and processing behaviour of pulse-derived ingredients, while laboratory twin-screw extrusion enables the investigation of texturization for meat analogue production. [1] These examples highlight the importance of assessing ingredient functionality alongside composition.

Rotational rheometry extends this approach through controlled measurements of flow behaviour and temperature-dependent structural changes. Starch pasting measurements illustrate how heating and cooling profiles reveal differences in viscosity development, breakdown and final consistency, providing a basis for ingredient selection and subsequent investigation of starch–protein formulations. [2]

Finally, soft-contact tribology complements bulk rheological measurements by probing friction and lubrication under conditions relevant to oral processing. A confectionery case study illustrates how samples with relatively similar viscosities can exhibit distinct frictional responses, demonstrating the additional information provided by tribological testing. [3]

Together, these methods establish a framework connecting processing behaviour, structural development and lubrication. This approach can support the formulation of alternative-protein and hybrid foods, while providing instrumental insights relevant to mouthfeel that complement sensory evaluation.

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