Dare 2 Change

INNOVATION DRIVEN AGRIFOOD BUSINESS

Abstract Book

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Dear Colleagues,

It was with great pleasure that we welcomed you in the second edition of Dare2Change conference, in the beautiful city of Porto.

This international scientific conference, co-organized by INIAV, Colab4Food and PortugalFoods, aims bridging the knowledge between academia and industry in the agri-food sector.

Great challenges such as the pandemic, war and climatic changes have brought the urgent need to find solutions to produce more food of high nutritional quality and safer, reduce waste based on reuse and circular economy, optimization of technological processes in order to guarantee food quality and increase food shelf life. We have a long way to go, but the excellence of Portuguese academia in this area, committed to work closely to Food industry will boost the prompt development of valid solutions and their fast introduction on the market that will greatly favor the Portuguese industry at international level.

The scientific committee created a program with sessions on emerging topics, where participants had the opportunity to discuss their latest findings in many areas of agri-food sector, encouraging networking opportunities.

We thank the sponsors for their commitment and support, essential to guarantee the success of Dare2Change.

This Book of Abstracts, contains more than 160 abstracts, presented as posters at the 2023 edition of Dare2Change. This number greatly surpassed the previous edition. Abstracts are divided in the following themes: Food Development and Production; Health and Wellness; Sustainability and Circular Economy; Digitization and Industry 4.0.

We thank your presence at Dare2Change, which enriched the conference and it was a key factor for its success. We are looking forward to seeing you again in the next edition of Dare2Change!

The Organizing Committee,

MIGUEL TEIXEIRA

Scientific & Executive Director | Colab4Food

ANA SANCHES SILVA

Assistant Researcher | INIAV

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This publication brings together the abstracts of the poster communications presented at the Dare2Change 2023. All abstracts were evaluated by the Scientific Committee of the Conference.

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PROGRAM

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09h00 Sessão de Abertura

Sessão Inaugural

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Daniel Ramón Vidal, ADM

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10h00 Inovar usando LEGO® Serious Play
Paulo Malta, Innovsky

10h15 Cell cultured Milk – the upcoming evolution
Omri Alfasst, Wilk

10h30 MicroHarvest: Nature's protein unleashed
Luísa Cruz, MicroHarvest

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Luke Young, ALORA

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14h30 Fábrica 5 G da Sumol+Compal
Pedro Tété Machado, NOS

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José Carlos Lopes, NET4CO2

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Dare2Tech

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Marlos Silva, MC

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17h45 **Farewell Beer & Cider Experience**

Dare 2 Change

Abstracts of Poster Presentations

Dare 2 Change

Digitalization and Industry 4.0

[D&I4.0 01]

Intelligent Self-Adaptable Food Systems Towards Farm to Fork Sustainability

Martins RC[1,*], Barroso TB[1,2], Silva FM[1,3], Moura P[1], Pinho T[1], Rodrigues L[1],Cunha M[1,3], Santos F[1]

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Food degradation, waste, nutritional, sensorial and safety losses are a consequence of the dynamics of internal (physical, chemical, biochemical, and microbiological contamination) and external (climate, ecology, farming practices, storage, distribution conditions and human interaction) factors. The highly dynamic food quality and shelf-life information is not present in the state-of-the-art food systems: *i.* shelf-life is set by a static date independently of the distribution chain characteristics; *ii.* food logistics, such as demand forecast and replenishment systems do not take into account food quality; and *iii.* the lack of information on internal and external factors affecting the shelf-life of foods.
Disruptive technologies, such as Computational Shelf-life Dating (CSLD), provide a forecasting system for computing the chemical, biochemical, sensorial, and microbiological processes of individual packages [1-3], along the distribution chain [4-5], allowing the forecast of nutritional composition, sensorial properties, and safety along the distribution chain, including at the consumer's home [6-8]. CSLD is the first system to provide a dynamical shelf-life evaluation of foods for optimizing the food chain, allowing consumers to evaluate the nutritional value [8].
In the age of Artificial Intelligence (AI) and Internet of Things (IoT), food systems can be implemented taking into account a dynamical and self-adaptable shelf-life. Here are proposed non-invasive intelligent sensors, biosensors and intelligent packaging that provide information about external and internal factors, determining important nutritional and safety properties. The information can be used to update CSLD systems and AI coupled with food logistics, appliances and point-of-measurement devices that provide diagnostic information to adapt the distribution system and optimize the nutritional and sensorial quality to reduce food waste and provide an efficient food system [8].

Acknowledgments: Filipe Monteiro-Silva (Silva, FM) gratefully acknowledges the financial support provided by FCT (Portugal's Foundation for Science and Technology) within grant (DFA/BD/9136/2020).

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[D&I4.0 02]

Intelligent Photonics Reagent-less Point-of-Care for 'In-Situ' and Real-Time Veterinary Animal Production Diagnosis

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Animal health and welfare is of the outermost importance in animal production and derived food products. Current state-of-the-art demands wellbeing-concerned design of facilities, as well as veterinary care with early detection of diseases. However, high density in animal facilities challenges preventive measures, animal growth and health monitoring, with intrinsic decrease in production efficiency.

Herein we present a Point-of-Care (POC) device based on spectroscopy and self-learning artificial intelligence, capable of performing blood, serum and urine analysis without the use of reagents or consumables [1,2]. The POC uses a plug-in and re-usable capsule system taking less than a drop of sample (< 10 µl), with diagnosis in less than 1s. The POC system is controlled by an Internet of Things (IoT) software assessable by any mobile phone. Recorded spectra are immediately processed to provide information on hemograms [1,2,3], and clinical chemistry [4,5].

Spectroscopy POC is ideal for veterinary medicine field work at animal production facilities, as this technology does not use reagents or consumables and is not affected by climate conditions. Moreover, the high-throughput capacity allows fast, accurate and inexpensive screening of several animals, paving the way for innovative POC herd diagnosis and effective public health measures.

Acknowledgments: Filipe Monteiro-Silva (Silva, FM) gratefully acknowledges the financial support provided by FCT (Portugal's Foundation for Science and Technology) within grant (DFA/BD/9136/2020). Rui C. Martins acknowledges Fundação para a Ciência e Tecnologia (FCT) research contract grant (CEEIND/017801/2018).

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[4] US10209178B2 Optical system for parameter characterization of an element of body fluid or tissue.
[5] EP18187384.5 A Method and apparatud for characterisation of constituents in a physical sample from electromagnetic spectral information

[D&I4.0 03]

Multimodal vineyard segmentation using deep learning

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Automation and robotics in agricultural domains will be relevant to face upcoming challenges with cost and labour scarcity. A large-scale deployment of automation, including robot systems, will additionally accelerate the transition towards more productive and environmentally sustainable farming practices. Robotic perception, a key component of robots, tend to be especially sensitive to "field" conditions therefore, the need for robust solutions such as fusing multiple modalities (a.k.a. multimodality) seems to be a promising direction to pursue. In this work, we propose MultiSegNet, a multimodal DL-based semantic segmentation approach for vineyard recognition. MultiSegNet is based on the SegNet [2] but,instead of having only one input branch, our network has two input branches: one for the RGB and another for the Digital Surface Model (DSM) modality. Each branch has an encoder that maps the inputdata to a features space, which is jointly trained using a supervised setting based on two losses. The first loss measures the binary cross entropy between the prediction mask (p_m) and the ground-truth mask (y_m): $\mathcal{L}_m = (p_m, y_m) = -y_m log p_m - (1 - y_m) log(1 - p_m)$, where $y_m \in [0,1]$. The second loss measures the similarity between the features of the RGB modality (h_x) and the features of the DSM modality (h_y): $\mathcal{L}_f(h_x, h_y) = 1 - \frac{h_x h_y}{max(\|h_x\|_2 \|h_y\|_2, \epsilon)}$, where ϵ is a margin value. The total loss $\mathcal{L}_t$ is then the weighted sum of the two losses $\mathcal{L}_t = \alpha\mathcal{L}_m + (1 - \alpha)\mathcal{L}_f$, where α is the weight which is tuned based on empirical studies. Thus, the network is simultaneously optimized to generate mask predictions and to learn similarities on both modalities. Empirical results indicate that this additional optimization step leadsto higher segmentation performance. As for the fusion of the feature vectors, we specifically studied three fusion operators: element-wise sum, element-wise product, and channel-wise concatenation - asshown in Fig. 1. The experimental evaluation of the proposed MultiSegNet was conducted on the GreenAI [1] dataset. This dataset is an aerial vineyard dataset of three different vineyards with RGB, DSM and multispectral data. The results, reported in Table 1, show that fusion by itself does not improveperformance, but adding the additional similarity loss the elementwise product (i.e., ElemPro) approach outperformance the baseline. Besides Vineyards case, the proposed approach finds applications in other agriculture domains e.g., Maize and crop monitoring.

Table 1: Intersection over Union (IoU) scores of the baseline and proposed models, trained and tested on theGreenAI dataset [1] with a split of 70-30, respectively.

SegNet (*baseline*)			0.82	
		ADD	Concat	ElemPro
MultiSegNet	Similarity Loss	0.81	0.77	**0.87**
	No similarity Loss	0.76	0.79	0.75

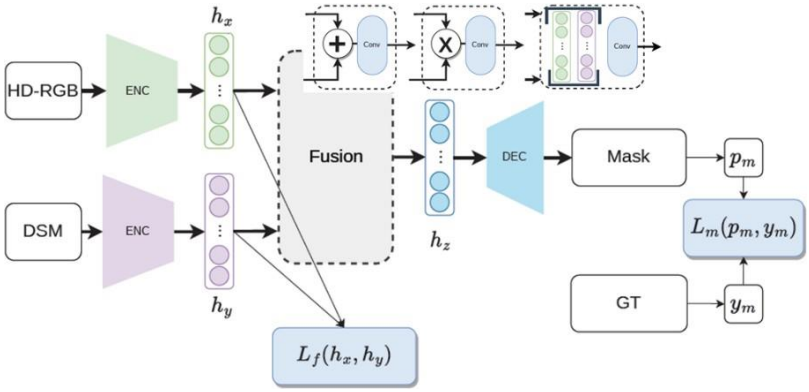

Figure 1: Block diagram of the proposed MulitSegNet approach.

Acknowledgments: This work has been supported by the project GreenBotics (ref. PTDC/EEIROB/ 2459/2021), founded by Fundação para a Ciência e a Tecnologia (FCT), Portugal. It has been also partially supported by FCT PhD grants with reference 2021.06492.BD.

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[D&I4.0 04]

Preliminary approach based on machine learning algorithms for commercial classification of rice types

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The quality control in food industry is extremely important at every stage of production, beginning with estimation of raw agricultural materials and monitoring their quality during storage, estimating food quality during the production process and of the final product's, as well as the determination of the authenticity, varieties sorting, and the detection of adulterants [1].

Rice (*Oryza sativa*), being one of the most produced and consumed cereal crops in the world, is widely used in food processing being fundamental to understand the properties of rice grains in order to control better production processes [2]. Based on the results of TRACE-RICE project, twenty-one varieties of rice, from 5 commercial types, were assessed by biochemical properties, agronomics traits, Rapid Visco Analysis (RVA), texture profile analysis parameters (TPA), cooked rice hardness and water absorption parameters. Classification procedure was carried out using several machine learning tools. The principal component analysis (PCA) was characterized by PC1 – 29.6%, PC2 – 17.6% and an explained variance of 99.1%. Several clusters were created, showing that the rice commercial types are defined according to its specific characteristics. The partial least-squares-discriminant analysis (PLS-DA) methodology allowed to obtain a significant score of rice type classification. The cross-validation for the model showed a cross-validation rate (88%), and accuracy (86%). The external validation for the model was characterized by non-error rate (97%), accuracy (94%), and a not-assigned samples (14%), being considered a significant value for classification step.

According to these preliminary results, robust classification models were developed using different machine learning algorithms based on biochemical, processing, and agronomics traits. The models obtained are suitable for commercial classification of rice types, with high accuracy, allowing to the producers, traders, and consumers to know with a promptly and safe mode the suitable rice grain, being considered an interesting advance to industry and consumers.

Acknowledgments: Funding for this research has been received from TRACE-RICE—Tracing rice and valorizing side streams along with Mediterranean blockchain, grant no. 1934 (call 2019, Section 1 Agrofood) of the PRIMA Program supported under Horizon 2020, the European Union's Framework Program for Research and Innovation, and Research Unit, UIDB/04551/2020 (GREEN-IT, Bioresources for Sustainability).

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Dare 2 Change

Food Development and Production

[D&PA 1]

Unfiltered Quince vinegar development and analysis: Meeting the rising demands on natural fermented products while using a climate-resilient fruit

Marlene Mota[1,2], Mário Cristóvão[1,*], Alexandra Camelo[1], Mafalda Resende[1], Cristina Miguel[1], Cátia Baptista[1], Inês Brandão[1,3], Christophe Espírito Santo[1,3]

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Quince (*Cydonia oblanga*) is a crop that can withstand extreme conditions like drought[1] or floods[2]. Although not very popular as a fresh fruit due to its hard texture, acidity and astringency, quince-based products such as jams or jellies like quince cheese are considered traditional products in Portugal [3]. Food innovation using quince as a basic ingredient, combined with strong dissemination, will foster greater recognition and appreciation for this culture. In addition, the market demands more sustainable, natural and functional foods which, together, envisage a lower environmental impact and healthier diets. Consumption of naturally fermented drinks like vinegar has become popular, with many claiming these products have numerous health benefits[4], although sometimes backed by limited scientific evidence.

The work describes the development and subsequent analysis of an unfiltered quince vinegar, a naturally fermented product with no preservatives. Three quince cultivars (n=40) were analysed and the one with the higher acidity (12.34± 2.45 g malic acid/L), cv. “Portugal”, was selected to improve the vinegar´s production.
To obtain a purer mother of vinegar, a cellulose biofilm composed of yeast and bacteria, two fermentations were carried out. Only the second formed biofilm was used in the final product. The other two ingredients used were quince and sugar.

The final product reached a pH of 3.08, 5% acetic acid and 0% alcohol, meeting the Portuguese regulations[5]. Finally, when subjected to sensory analysis (n=20), 75% of consumers would buy this product, if available in the market.

Acknowledgments: CULTIVAR (CENTRO-01-0145-FEDER-000020); Fusilli (European Union’s Horizon 2020 under grant agreement No. 101000717)

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[D&PA 2]

Orange snacks as a solution for orange surplus in Portugal

Mário Cristóvão[1,*], Alexandra Camelo[1], Ana Martins[1], Ana Silveira[1], Ana Riscado[1], Ana Rodrigues[1], Cátia Baptista[1], Helena Beato[1], Vanessa Vasconcelos[1], Luisa Paulo[1], Inês Pitacas[2], António Moitinho Rodrigues[2,3], Christophe Espírito Santo[1,4], Inês Brandão[1,4]

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In the summer of 2022, Portugal experienced a downfall of the price of oranges, mainly due to pressure of the agricultural commodity market and the change in trade dynamics in Europe due to the war in Ukraine. The price of this fruit fell exponentially when oranges that normally go to the East were all brought to European markets. This caused some Portuguese producers to abandon much of their production, leaving about 93% of their crop rotting on trees[1].

One way to add value to a product is to transform it into something new. Dehydration can increase the value of the final product and extend its shelf life[2], but in the case of oranges, it is mostly used to obtain a powder that can be used as an ingredient[3]. The aim of this work is to produce a versatile, ready-to-eat orange crisp. Oranges are the only ingredient of this snack that can be presented in a variety of ways, such as a crunchy snack, an edible cake topping or as an ingredient in drinks such as gin or other drinks.

The product was fully characterised in terms of nutritional value, water activity (a_w), microbiology and sensory analysis. The result is a high-energy product with no added sugar, no fat and no salt. The final product has a low a_w value (0.238), which limits enzymatic and chemical degradation reactions and microbial development. In the sensory analysis (N=66), the snack received an overall score of 8±1 (on a scale of 1 to 9) when asked if the product is something they would buy, the acceptance rate was of 96%.

Acknowledgments: CULTIVAR (CENTRO-01-0145-FEDER-000020)
Fusilli (European Union's Horizon 2020 under grant agreement No. 101000717)

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[D&PA 3]

Postharvest valorization of fruits, vegetables and by-products

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Current consumption trends demand sustainable, safe and healthy products with a long commercial shelf-life [1]. Simultaneously, the existing global commitment to reduce climate change and food wastepromotes the need for new processes and technologies. Pulsed electric fields (PEF) technology promotes cell electroporation, a non-thermal effect that creates pores in cell membranes, and has shown great efficiency in microbial inactivation and as a processing technology [2,3]. The application of short duration PEF (µs–ms) in food results in minimally processed products, with sensory and nutritional characteristics similar to their fresh equivalents [2]. The main objective of this study was to evaluate theimpact of the synergy between PEF and other technologies such as ohmic heating (OH) and freeze- drying in the search for sustainable food processes. This study was carried out in partnership with companies and universities, particularly using low-caliber fruit and vegetables, such as: strawberries, potatoes, carrots and apples, in addition to fig leaves. Microbiological, shelf-life, texture, microscopy, extraction yields and economic viability analyses were performed. The results show that PEF at 10 kV/cm, reduced inoculate *E. coli* in fruit puree by 5.8 Log CFU/g, and ensured the microbiological stability for at least 30 days. The synergy of PEF and OH reduces energy consumption and pasteurization temperature in juices. In vegetables, PEF induces a loss of turgor pressure and tissue softening, resulting in a smooth cut. Regarding to dehydrated products, when PEF is used before freeze- drying, energy consumption can be reduced by up to 40% and drying time between 20-35%, depending on the product. As far as PEF-assisted extraction is concerned, a yield between 14–18% was obtainedfrom fig leaves. In conclusion, the impact induced by PEF is important for the industry in terms of economic viability and opportunities to improve the food quality and process sustainability.

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[D&PA 5]

The distribution of contaminants in foods according to the scientific literature data mining and machine learning

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Know the patterns of contaminants distribution in raw materials and food items is of major relevance to food industry prevent their presence in the final processed foods. External factors, such as, production conditions, climate changes, foods availability, legislation parameters, among others, changed those patterns in the last decades. Therefore, industry needs to be updated concerning, not only patterns but also trends in the distribution of contaminants in foods. Scientific literature provides a great amount of valuable data, however, information is disperse and summarise it manually is not feasible. To tackle this gap, our goal was to implement a systematic organization of existing knowledge concerning food contaminants in highly produced/consumed food items and use predictive modelling and machine learning (ML) techniques to identify the patterns of contaminants distribution in a great variety of foods and the trends of their distribution in the last 2 decades.

Literature mining accomplished by FoodMine's code [1], with some modifications was used. Eighty foods were selected according to its relevance in FAOstat database and and named according to FoodEx2. PubMed Advanced Search Builder was used to determine the best terms and search strategies. A total of 96 food contaminants were searched in 80 raw materials/foods, including, heavy metals (mercury, lead, cadmium, arsenic), polycyclic aromatic hidrocarbons, dioxins and dioxin-like PCBs, pesticide residues, disinfection by-products, heterocyclic aromatic amines, and natural toxins (mycotoxins). A PubMed search done with all the selected terms would return almost 2 billions entries, which were reduced by FoodMine code to 26,697 and by further ML to 1,887 articles, which were manually reviewed. Finally, 438 articles were effectively used to extract data, covering the period between 2000 and 2022, to build a database of contaminants content In foods and estimate their prevalence. To predict the patterns of contaminants distribution, the 80 food items were classificed in 12 groups (most of them according to hieralchical level 1 of FoodEx2). Mathematical modelling was performed on data from Europe and North America and compared with data from the whole world, and showed different patterns of contaminants distribution that are a reflex of different regulations across world. This is a valuable information on quality and safety control for industries that acquire raw materials from other regions than Europe and North America.

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[D&PA 6]

Hops as a promising Clean Label preservative in food

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Hops, a beer ingredient, have been described with antioxidant and antimicrobial activities, related to the compounds such as alpha acids (responsible for the beer bitterness), beta acids, and xanthohumol. Proprieties that can make the hops a clean label alternative to guarantee oxidative and microbiological stability in other food products, outside the beers. However, beyond the bitterness, hops have an essential oil fraction containing a wide variety of volatile compounds that adds aromatic characteristics to the plant.

In this work, it was proposed to verify the potential of hops to be used as a natural preservative agent in emulsified sauces (ketchup, mustard and mayonnaise). The work was developed in collaboration with the Casa Mendes Gonçalves industry. The objective was to verify the possibility of adding hops in sauces, in low concentrations, not able to promote changes in sensory characteristics, but high enough to exert protective effects. The selection of hops was done based on their bioactive composition, including alpha/ beta acids (spectrophotometry), xanthohumol content (HPLC-UV-DAD), and antimicrobial properties (disc plate method). Then, hops were incorporated in sauces, and sensory analysis (quantitative descriptive and ranking test) with a trained was done.

A hops variety with low alpha acids, moderate to high beta acids and xanthohumol content was selected. The concentrations of 0.1% in ketchup, 0.2% in mustard, and 0.06% in mayonnaise were considered as the maximum amounts of selected hops to be incorporated without noticing organoleptic changes. The approach highlighted the potential of hops to be used as a natural preservative of sauces. Since, at the found concentrations, according to the chemical composition and literature data, hops have enough amounts of bioactive compounds to exert antioxidant and antimicrobial activities against a wide range of bacterial species. Including microorganisms that frequently cause concern in these products.

[D&PA 7]

Manufacturing cultured meat through a scalable and cost-effective bioprocess

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In recent years, there has been an increasing interest in cellular agriculture, an emerging field that aims to overcome most issues associated with livestock meat consumption, such as its environmental impact, risk of foodborne diseases and animal slaughtering. Different approaches have been attempted to produce cultured meat, but high production costs and scalability limitations have hampered this technology. Bioprocess scale-up is crucial to ensure the product's economic viability and availability, so key factors must be optimized, such as the cell source, bioreactor configuration, culture medium, scaffolding and biomaterials to support cell proliferation and achieve the desired properties. To address this, our work focused on optimizing cell proliferation and differentiation into relevant cell lines under static conditions. Mesenchymal stromal cells (MSC) were chosen as the starting cell line due to their high proliferation potential and ability to differentiate into myocytes (the principal constituent of meat) and adipocytes (which contribute to the organoleptic properties of meat). MSC isolation from bovine umbilical cord was successfully performed using explant culture, and the resulting cells were characterized according to the expression of key MSC markers (CD105, CD90, CD29, CD44) and expanded under different 2D culture conditions. Future studies will focus on optimizing cell proliferation and differentiation, both under static and dynamic conditions. To achieve the latter, it will be necessary to establish a bioreactor system and develop appropriate microcarriers for bovine MSC expansion and differentiation. Thus, food-grade biomaterials will be optimized concerning size, surface charge, morphology and porosity, among other key properties, and assessed according to the resulting organoleptic properties of the cultivated meat product.

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[D&PA 8]

Lipid profile of fish by-product oils obtained by microwave-assisted extraction and Soxhlet

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The fishing industry produces large quantities of fish by-products every year, currently little explored by the agri-food sector. However, the upcycling of these natural resources has been promoted by the generally high lipid content they have and by the emergence of more sustainable extraction techniques, but still little explored to obtain lipid fractions from fish by-products.

Therefore, this work aimed to obtain oils from fish by-products by microwave-assisted extraction (MAE) and Soxhlet extraction (SE) and to characterize their lipid profile. Category 3 fish by-products, supplied and considered standard by the processing industry (ETSA Group), were freeze-dried and subjected to the following extraction conditions: 17 min of irradiation at 750 W, at a solid/liquid ratio of 70 g/L, for MAP and 6 h of extraction at 80 ºC, at a ratio of 20 g/L, for SE; hexane was the extraction solvent used in both methods [1]. The oil yield was determined gravimetrically and the fatty acid profile was analyzed by gas chromatography with flame ionization detection (GC-FID), after a derivatization process performed to obtain fatty acid methyl esters.

The MAE gave an oil yield similar to that of SE (about 18 g/100 g dw). The lipid profile consisted mostly of unsaturated fatty acids, due to the high levels of oleic, docosahexaenoic (DHA) and linoleic acids, which were not significantly affected (p <0.05) by the extraction methods. These oils will be of interest for animal feed formulation.

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[D&PA 9]

Nutritional quality of fresh-cut watercress stored under modified atmosphere packaging

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Watercress (*Nasturtium officinale* R. Br.) is a nutrient rich perennial plant of the Brassicaceae family highly appreciated in the Mediterranean cuisine. It is eaten raw in salads, soups and other recipes and used in folk medicine due to its medicinal and therapeutic properties [1,2,3].

Due to its reduced shelf-life, the effects induced by different packaging systems on physical, chemical and functional quality parameters were evaluated and compared in order to select the most efficient treatment for shelf-life extension. Wild samples were gathered in Bragança region, rinsed in tap water and a portion was immediately analyzed (control). The remaining fresh material was packed in conventional (air- and vacuum-packaging) and non-conventional (N_2- and Ar-enriched modified atmospheres (MAP)) systems and stored at 4 ºC for 7 days. After assessing the effect on individual parameters, a linear discriminant analysis provided an overview of the suitability of the tested packaging system in maintaining the fresh-like properties of the control samples throughout the storage time. The complete individualization of the four packaging systems was observed, being the air-packaged samples those to present the most dissimilar profiles from the control, followed by the N_2-enriched MAP ones. In turn, Ar-enriched MAP was the best option to preserve the overall quality. Nevertheless, some of the observed changes were advantageous for the samples' functional properties.

Overall, this study highlighted Ar-enriched MAP as the best option to preserve fresh-cut watercress during refrigerated storage. Further studies are necessary to evaluate the effects on physiological and sensorial parameters.

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[D&PA 10]

Assessment of coffee adulterations by gas chromatography and electronic tongue

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Coffee is an economically relevant commodity worldwide, being its popularity greatly linked to its pleasant aroma and flavour. However, coffee is frequently adulterated for economic gains through the incorporation of low-cost raw materials in coffee powder, such as coffee husks (also known as cascara from the coffee cherry) [1]. Thereby, there is a need to adopt fast and reliable methodologies for the detection of coffee adulterations to ensure coffee quality. This work aims to evaluate the feasibility of using headspace solid-phase microextraction coupled to gas chromatography/mass spectrometry (HS-SPME/GC-MS) and chemical sensors (electronic tongue) for the detection of coffee adulteration promoted by coffee husks. Commercial medium roast Colombia coffee was admixed with coffee husks at different concentrations (2% to 20%, w/w). A total of 72 volatile compounds were determined in all samples. From these, b-damascenone and linalool oxide, both related to the secondary metabolism of the coffee plant, were detected only in coffee husks and adulterated samples, suggesting that these compounds can be used as adulterant markers in coffee brews. Besides, both volatile composition and electronic tongue responses were used for constructing calibration models for the prediction of the coffee husk concentrations added to coffee. Calibration models were calculated using Partial Least Square regression with leave-one-out validation. The results confirmed the feasibility of HS-SPME/GC-MS for adulterant detection down to 2% w/w of coffee husks added, while the chemical sensors allowed its detection down to 5% w/w. This study showed that HS-SPME/GC-MS and chemical sensors can be used as simple and sensitive tools that can be easily applied at industrial level to detect adulterations in coffee brews.

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[D&PA 11]

Nutritional characteristics and minerals of Lardosa cowpea landraces: a strategic legume species for the future

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Cowpeas have been cultivated at least since the 8th century BC. [1; 2]. This grain legume (*Vigna Unguiculata* L. Walp.), one of Africa's natural food gifts to the world, is characterized by its high nutritional value, ability to fix nitrogen and great tolerance to drought. The tolerance of this legume to low fertility soils, high temperatures and scarce water regimes makes this one of the most resistant crops to adverse edaphoclimatic conditions. It is certainly a very important culture in southern European countries like Portugal.

For this study, three types of cowpea landraces, “black face”, “green face” and “rice” were analysed. Coming from the region of Lardosa, a parish in the municipality of Castelo Branco, these beans are used for both human and animal consumption. Cowpeas, cooked in boiling water, were nutritionally characterized. Besides, the minerals were determined in cowpea grains, in the soaking water and in their cooking water. Sodium, copper, iron, manganese, zinc, phosphorus, calcium, magnesium and potassium were measured.

Overall, the Lardosa cowpea grains ranged between 63.8 - 66.1% moisture, 1.47 - 2.45% fibre, 8.22 - 9.46% protein, 0.69 - 0.78% ash and 22.43 - 22.95% carbohydrates. Energy values varied from 571 kJ (134 kcal) to 599 kJ (141 kcal) per 100 g. The pH and acidity range of values were 7.16 - 7.25 and 0.95 - 1.02 mEq/100 g of sample, respectively.
The performed analysis showed the nutritional potential of the three cowpea landraces, contributing to a greater knowledge of these agro-food resources that will probably become one of the foods to take into account in the near future, given their capacity to thrive in the face of climate changes.

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[D&PA 12]

Kansei Engineering methodology to support the development of an innovative ready to eat algae soup

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Technological advances have tremendously impacted product development, driving industries to develop new products and introduce them into the market. Marketing tools are indispensable to promote the creation of previously untapped market niches and to correctly develop and introduce a new product.

In the present study, *Kansei* Engineering (KE), a consumer-oriented marketing methodology that studies the emotional aspect of a product design through statistical and mathematical models, was implemented to develop an innovative ready-to-eat algae soup.

This product is envisaged for the near future and KE will help facilitate its market entry and further success. The methodology from Schütte [1,2,3] and Marco-Almagro [4,5] was further extended, including a free word association survey, to obtain *Kansei* words directly from consumers, and a word categorization to include word frequency calculations and assist the selection of high-level *Kansei* words. This choice was made through meetings with both experts and a rice company, resulting in six words selected: 'Appealing', 'Convenient', 'Informative', 'Natural', 'Nourishing' and 'Sustainable'. Proper statistical tools, including models QT1, OLR, BLR and MRA were used to reach guidelines for the final product design, based on the analysis of the relationship between product properties and the emotions perceived by consumers.

Results show that the company should attempt a combined optimisation of the different *Kansei* words, with the resulting packaging presenting a window on the top sleeve, the soup texture should be with algae pieces, the preservation method should be refrigerated, and the labelling should include additional quality claims.

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[D&PA 13]

Fractionation of edible insect *Tenebrio molitor* – valorisation of high-value products

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The goals of this work were to develop extraction techniques to obtain protein concentrates from *Tenebrio molitor* larvae.

Two different protein extraction methods were developed, based on isoelectric point precipitation or membrane ultrafiltration. For both methods, dried *T. molitor* larvae was defatted with the Soxhlet method with ethanol as a solvent. The defatted fraction was then homogenized in a NaOH solution with the homogenate being recovered and centrifuged. The supernatant (S) and the pellet fractions were recovered. The pellet was submitted to acid/alkali hydrolysis followed by deacetylation to obtain chitosan.

For the isoelectric point precipitation method, the recovered supernatant pH was modified to 4.546 and the precipitate was centrifuged. The pellet fraction was freeze-dried (IP). For the membrane ultrafiltration, the supernatant was filtrated with a 50 kDa membrane with the retained (> 50 kDa) and filtered (<50 kDa) being recovered and freeze-dried. The fractions were characterized for their protein content, protein profile (SDS-PAGE and FPLC) and techno-functional properties (colour, foaming properties, water/oil absorption capacities and emulsifying properties).

Both the IP and the > 50 kDa fraction had protein contents above 80%. The >50 kDa fraction had a very similar protein profile to the supernatant, while the IP fraction was composed of protein with higher molecular weight. The >50 kDa fraction had higher *L** (lightness) and *b** (yellowness) colour than the IP fraction or the defatted or oven-dried samples. Additionally, the samples presented better techno-functional properties than the dried or defatted sample and the >50 kDa fraction had better properties than commercial protein concentrates (whey protein or pea protein).

The protein extraction based on ultrafiltration has undergone a patenting process [1] and led to a protein concentrate with high purity and acceptable techno-functional properties which can function as an alternative to the more common method based on isoelectric point precipitation.

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[D&PA 14]

Isolation, identification and whole genome sequencing of one *Pseudomonas* spp. from raw milk responsible for discolouration in different culture media and cheese

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In recent years, artisanal foods and the use of raw milk for cheese-making are gaining popularity among consumers worldwide [1]. In Portugal, traditional agrifood economic activities remain crucial for local and regional economies. Here, most traditional cheeses are made with raw ewe's and goat's milk [2]. Visual defects such as the appearance of discolouration on the surface of cheeses may, however, generate distrust in the consumer, leading to economic losses and to the depreciation of this endogenous resource [3,4]. Hence, it is important to understand the cause of visual defects and use appropriate preventative methods.

In order to determine whether the causes of colour discolouration are due to the presence of any particular microorganisms, culture plates to determine *Escherichia coli* ß-glucuronidase positive, mesophilic germs, *Pseudomonas* spp. psychotropic germs, moulds and yeasts were made from 406 samples of milk and 56 of cheese, over 2,5 years.

One particular isolate from Nutrient Agar medium showed an intense brown pigmentation.
The isolate, identified as *Pseudomonas* spp., was subsequently isolated and purified for further study. DNA was extracted and whole genome sequencing was performed using long-read Next Generation Sequencing (Oxford Nanopore Technologies). The ability to produce discolourations was screened with the *Pseudomonas fluorescens* multi-locus sequence typing (MLST) tool.

To understand the evolution of the pigment developed by this particular *Pseudomonas* spp. isolate, an in vitro study was designed to recreate the colouration development in different media (n=3): Agar-Cheese with and without tyrosine, Luria Bertani Agar, King B and Nutrient Agar at different temperatures. A brown pigmentation associated with the isolate was observed in every medium.
Simultaneously, a trial was made to simulate real cheese fabric conditions with raw sheep milk, and 45 days of ripening (n=10). Noteworthy, a strong blue pigmentation was evident on the surface of inoculated cheeses, but not on negative controls.

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[D&PA 15]

Quality of Beira Baixa “Queijo Amarelo” (Yellow Cheese)- P.D.O. Effect of Production Season on Microbiological Quality

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Beira Baixa “Queijo Amarelo” (Yellow Cheese) – Protected Designation of Origin is a cured cheese, semi-hard or semi-soft, slightly yellowish, obtained by slowly draining the curd after coagulation of raw sheep's milk, on its own, or a mixture of sheep's and goat's milk, by the action of animal rennet. The P.D.O. requires the cheese to be produced in accordance with certain rules, namely: milk production conditions, milking hygiene, milk conservation and product manufacture. The microbial component is fundamental for determining the quality of cheeses, with dynamics of interaction between the different groups of microorganisms naturally present being a determining factor in the final quality of the cheese produced. In addition to all the factors inherent to the quality of the raw material, production and processing conditions, several authors point out that the production period can influence the microbiological quality of cheeses. The present work aimed to evaluate the quality of the Yellow Cheese from Beira Baixa and to verify if there is an effect of the production period on the microbiological quality. Over 2,5 years, milk and cheese samples were collected and analyzed. In milk, they were enumerated by counting on selective agar (Coliforms, Molds, *Escherichia coli* (*E.coli*), Yeasts*, Pseudomonas*, Microorganisms at 30ºC and Psychrotrophic Microorganisms), in cheese (Lactic acid bacteria (LAB), Coliforms, Molds, *Enterobacteriaceae, E.coli, Staphylococci*, Yeasts and Psychrotrophic Microorganisms. Later, results were grouped by season (Autumn, Winter, Spring and Summer), in order to verify if there are microbiological differences in the quality of the cheese in the different seasons.
Based on the results, we conclude that the analyzed milk and cheese are in accordance with current legal microbiological standards. In milk samples, Pseudomonas and Psychrotrophic Microorganisms are predominant when compared to the other parameters. In cheese samples, LAB and Psychrotrophic Microorganisms have the highest counts. In general, there is a lower count of microorganisms in summer, in both milk and cheese.

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[D&PA 16]

Consumer behaviour and market trends, what to expect...

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Understanding consumer behavior, its preferences, needs and tastes, should be taken account in the new product development strategy. The integration of R&D and Marketing allows us to interpret new market trends and design products that will contribute to innovation success[1].
Colab4Food grouped macrotrends for the next year that will impact the market, transversal to all Food & Beverages (F&B) categories: Consumer Connection, Health & Wellbeing, Sustainable Food Systems, and Digitalization. More than ever, the "value for money" of a product is at the heart of consumers decision-making, although they don´t want to give up of satisfaction and pleasure, new experiences, small luxuries, personal wellbeing or core values. Promoting healthier eating, with a focus on mental wellness, energy and cognitive boost, ensuring daily nutrients intake at affordable prices, will increase pressure on the food industry. The challenges include optimization of plant-based and functional products, with natural ingredients, that are better for me and for the planet. Consumers are also increasingly attentive to the whole supply chain and are concerned about their lifestyle and the impact of their food on the planet, asking brands to be transparent and demonstrate that their actions are ethic and are committed with the environment, citizens and animal welfare. Digitalization will increase transparency and circuits agility, allowing the consumer to better understand the products and their processes and lead them to have mixed and entertaining experiences, which could bring new opportunities for brands.
In "Alcoholic Beverages", "Bakery", "Dairy", "Meat substitutes", "Meat, Fish & Eggs", "Sauces & Seasonings", and "Soft Drinks" categories, will be given practical examples of products positioning and claims[2] (e.g. "Convenience - Packaging or Ethical - Packaging") aligned with macro and consumer trends. Global uncertainty, affordability of products, nutritional and emotional benefits, and instant gratification, will drive the design of new F&B products this year.

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[D&PA 17]

Study of the suitability of different protein sources on the physical characteristics of protein extrudates obtained by high-moisture extrusion cooking.

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The production of palatable meat analogues using high moisture extrusion cooking is a complex process that depends on both the properties of the protein ingredients and the extrusion conditions (Sandoval et al., 2019). This study aims at gaining a better understanding on the texturization and physical properties of protein extrudates obtained by this technique using different protein sources.

For this purpose, the behaviour after high-moisture extrusion, of different alternative proteins such as hemp and insects, when incorporated as a secondary ingredient in a soy-based formula and a pea-based formula, was compared. In addition, physical characteristics of the control proteins (soybean and pea) were also studied individually and in combined formulas. Extrusion experiments were performed using a co-rotating and intermeshing twin extruder. To describe the physical characteristics of the extrudates, texture tests (longitudinal and transverse) and microstructure analysis were carried out.

The results of the maximum shear force measurements in longitudinal and transverse direction, indicated that pea control sample (100% pea protein) presented significant higher maximum shear force than soy control sample (100% soybean protein). Furthermore, in longitudinal tests, the addition of insect protein showed a reduction in maximum shear force in both cases. On the contrary, the addition of hemp protein presented an increase in maximum shear force. The mixture 50% pea and 50% soy indicated a maximum shear force highly superior to each one its own. Microstructure analysis showed that the control proteins, individually and mixed together, generated structured and homogeneous matrices. Additionally, insect protein incorporation resulted in more heterogeneous and porous extrudates. Hemp protein added to soy increased the compactness, while in the pea formulation had the same effect as insect protein. Our findings show that soybean, pea and hemp are valuable raw materials for the development of fibrous structures by high-moisture extrusion cooking.

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[D&PA 19]

How dehydration affects the Strawberry Tree Fruit

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Strawberry tree (*Arbutus unedo* L.) it´s an endemic species of the Mediterranean region, and is widely present in Portugal especially in regions up to 800m with a mild climate[1].It was of great importance because of its ability to withstand forest fires and adverse climatic conditions[2]. The consumption of its fruit has benefits for human health, although it's a seasonal and perishable fruit, alternatives for the use of strawberry tree fruit is on demand.
Dehydration may be one solution, as it involves minimal cost and low environmental impact[3], and provides a new option for strawberry tree fruit consumption and reduces food waste[4].

This work aims to understand the dehydration of strawberry tree fruit, its kinetics, physicochemical and microbiological properties. The fruits had an initial moisture content of 67.7%±1.1, and dehydration occurs at 60 °C with an air flow of 1.2 $m.s^{-1}$ until equilibrium moisture content (0.9%±0.1).

The result of dehydration was ground in powder form, that can be a way to have this fruit available throughout the year.
A comparison was made between the fresh and the final product to understand if their characteristics are loss and which are gain. With the latter presented a low fat and saturated fat, high fibre content and antioxidant activity. It was a stable enzymatic development with a water activity of 0.263±0.032. The powder can be used as a healthy ingredient for the food industry and be an impetus for the dissemination of this fruit, as it's easier and more stable to transport and sell.

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[D&PA 20]

Vegetable oil quality: the added value of halophyte plants

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The halophyte plants, such as *Salicornia ramosissima*, are recognized sources of bioactive compounds (e.g., phenolic compounds, carotenoids), with potential antioxidant properties and consequent health promoting effects. With environmental advantage in saline environments, these salt tolerant plants accumulate in their roots, shoots and leaves minerals like sodium and potassium. Consumers are motivated to purchase these plants to use them as a gourmet alternative for salt with antioxidant properties [1].

During storage and especially during the frying process, the vegetable oils can be oxidized and present undesirable flavours. Therefore, natural alternatives to synthetic antioxidants are sought by the oil food industries [2]. In this study the dried halophytes, and their correspondent ethanolic extracts, were added to different vegetable oils (produced from maize, peanut, and sunflower) to evaluate the antioxidant effect of these plants and extracts in the oil's oxidation process during the frying process.

After characterization of the antioxidants in the halophyte plants and extracts (e.g., phenolic compounds and carotenoids) using spectrophotometric and chromatographic approaches, the enriched and corresponding non-enriched (reference) oils were submitted to several cycles of frying (seven cycles). The samples collected after each cycle were reanalysed for their antioxidant composition, colour, oxidation degree and lipids composition.

The volatile composition of the most promising enriched oil in antioxidant compounds was compared to the non-enriched oil before and after applying the frying cycles to tentatively identify the volatile compounds formed by oxidation of the oils during the frying process. The obtained results are relevant for the consumer and food industry worried with the quality of the vegetable oils used for frying purposes.

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[D&PA 21]

The high diversity in pulses' volatile composition: understanding the chemical basis for sensorial legumes acceptability / avoidance

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Pulses represent the most accepted food alternatives to animal protein, especially when combined with cereals. The inclusion of pulses in the diet improves the protein, fibre, minerals (e.g., potassium), and vitamins (e.g., folate) intake, as well as the bioactive composition in the diet. The consumers' acceptability of legume-based novel products depends on their taste and aroma. This last attribute is directly dependent on the volatile composition of the raw materials used in food processing [1].

Although phenolic compounds, saponins, and free amino acids have an impact on organoleptic sensations, volatile compounds are key players in the consumers' acceptance of legume-based products. The volatile compounds mostly associated with unpleasant odours in legumes result from the fatty acids oxidation [2]. Nevertheless, there is a lack of knowledge regarding the intra- and inter- diversity of the volatile composition in the different legume species.

To understand the volatile diversity in legumes, six different species (PV - *Phaseolus vulgaris* L., LS - *Lathyrus sativus* L., VF - *Vicia faba* L., PS - *Pisum sativum* L., CA - *Cicer arietinum* L., and LC - *Lens culinaris* L.), each represented by about 100 diverse accessions, were studied. The plants were cropped at the same location in Córdoba, Spain, with an Autumn-Winter sowing for the cool season LS, VF, PS, CA and LC, and a Spring-Summer sowing for PV, and their corresponding whole flours were characterized by SPME-GC-MS/MS.

The results showed a huge intra- and inter- diversity in the volatile composition of the different species. Among the autumn-winter sowing species, the LS stood out by their lower percent area of limonene, α-pinene and decanal, associated respectively, to citrus, earthy and fatty odours. In the spring-summer sowing accessions of PV, compounds such as the phenylethylic alcohol contributed to the sweet, and fresh aroma typical of *Tarrestre* bean variety.

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[D&PA 22]

Phenolic composition and antioxidant capacity of pea- and oat-based expanded snacks produced by extrusion

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Cereals and pulses are complementary plant materials to provide a balanced amino acid profile and contributes to obtain healthier food products. Extrusion technology is commonly used in the food industry to produce expanded snacks. During extrusion, as consequence of the macromolecule's disruption, changes in physicochemical and nutritional properties of extruded products may occur.

An expanded snack based on a pea protein concentrate (PP), a pea starch concentrate (PS) and an oat beta-glucan-rich fraction (OF) was produced through low-moisture extrusion [1]. Moreover, local Portuguese products, such as, *Bravo de Esmolfe* apple (BE), grass pea (GP) and a red seaweed (Gracilaria gracilis - GG) were added to the main formulation (BM). The phenolic composition and the antioxidant capacity of the blends (non-extruded) and snacks (extruded blends) were evaluated.

The OF and the PS largely contributed to the final phenolic composition of the snacks and respective mixtures before extrusion processing. Higher amounts of p-hydroxybenzoic acid and hydroxycinnamic acids (e.g., caffeic acid, sinapic acid and ferulic acid) were provided by the OF and higher kaempferol content was supplied by the PS. More than 50% of the hydroxycinnamic acids and p-hydroxybenzoic acid were found in the bound fraction. The antioxidant activity was improved by the addition of *Bravo de Esmolfe* apple (BE) which contributed with higher contents of p-hydroxybenzoic acid, caffeic acid, quinic acid, catechin, procyanidin B1, 3-hydroxybenzoic acid and phloridzin to the original healthy snacks.

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[D&PA 23]

Impact of processing on antinutrients present in pulse protein concentrates and food products

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Pulses have been gaining interest as an alternative and sustainable plant protein source. Dry seeds are rich sources of proteins, energy, carbohydrates, fiber, B-vitamins, and minerals. However, pulse seeds also contain minor components, known as antinutrients, which include enzyme inhibitors, lectins, alkaloids, phytic acid, and some specific phenolic compounds like saponins and tannins. The existence of these components in the human digestive tract reduces nutrient absorption, pulses' digestibility, and nutrients' bioavailability [1,2]. Nonetheless, some of these antinutrients are reduced or eliminated during soaking, cooking, and processing [3].

Raw-materials, protein fractions and ingredients derived from pulses were used to produce protein concentrates [4], snacks [5] and texturized protein (to be further used in the formulation meat analogues). The impact of processing, namely dry fractionation, cooking and low-moisture extrusion, on specific antinutrients, such as trypsin inhibitors, total saponins and total tannins was evaluated.

Regarding protein concentrates obtained by dry-fractionation, results showed that in general trypsin inhibitors are concentrated in the protein fraction. Moreover, the respective cooked raw-materials and fractions demonstrated a reduction between 58 and 86% in trypsin inhibitors. The analysis of tannin content indicated that the dehulling step is not needed to remove these constituents when the interest is focused on the protein concentrate. Results showed no significant differences for the saponin content of the non-extruded and the extruded blend of a faba bean protein concentrate and an oat fraction. Regarding the trypsin inhibitors, a 93% reduction upon extrusion was achieved. Moreover, the tannins were not detected both in the non-extruded and extruded blends. A blend of pea protein, pea starch and an oat fraction were also processed to produce snacks and trypsin inhibitors were also reduced (33.6-78.3%).

Overall, cooking and extrusion reduced trypsin inhibitors. For saponins no significant differences were found between their content before and after extrusion.

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[D&PA 24]

Extraction of antibiotics from the egg matrix

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Human beings consume a daily dose of residual antibiotics from animal foods, plant foods and drinking water, as antibiotics are widely used in animal husbandry and easily transmitted to the environment and into the food chain. Of this daily dose, 10 to 15% comes from egg consumption [1]. The intake of small concentrations of these drugs concerns public health authorities due to the promotion of antimicrobial resistance, which is estimated to cause 10 million deaths a year by 2050 [2].

Thus, the objective of this research is to develop different methods of extracting antibiotics from eggs, determine which one is the best and promote food safety by providing a monitoring tool [3,4]. Blank samples were spiked with 64 antibiotics and then extracted with the following variations: 1 – Liquid-liquid extraction (LLE) with acetonitrile (ACN) and the analyte's separation by freezing; 2 – LLE with ACN and methanol and the analyte's separation by freezing; 3 – LLE with ACN; 4 – LLE with ACN and methanol; 5 – LLE with ACN and degreasing. The extracts were injected into the UHPLC-TOF/MS using the column Acquity HSS T3 2.1x100 mm, 1.8 µm particle size, and 0.1% formic acid and ACN as mobile phase.

The methods were successful in detecting 50 to 58 compounds, with the highest number of antibiotics detected by methods 3 and 5. Mean recovery rates ranged from 36.8% to 92.3% and methods 3, 4, and 5 were the most satisfactory for their mean recoveries greater than 70%. Thereby, it is concluded that the LLE with ACN includes a greater number of compounds than the ELL with ACN and methanol, regardless the degreasing stage has been carried out. However, degreasing contributes to a higher recovery rate because it results in noise and matrix effect interference reduction and generates better defined chromatographic peaks with a larger area.

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[D&PA 25]

Determination of Antibiotics in Animal Food products towards OneHealth Approach

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The abusive use of antimicrobials in various sectors such as agriculture, animal husbandry and human health has raised concerns from a One Health perspective. Misadministration of drugs, inadequate infection control, agricultural and animal residues are sources of contamination and allow the migration of both antibiotic residues and resistant bacteria for the triad: humans, animals and the environment, all suffer from antimicrobial resistance [1]. Focusing on consumer health, the European Commission created a regulation that establishes the maximum residue limits for pharmacological substances to be present in food of animal origin without representing a threat [2]. To find out whether a food complies with the regulated concentrations or that it does not contain prohibited pharmacologicalsubstances, quantification analysis of these analytes are carried out in the animal matrix, however, for the method to be validated, it must comply with a series of requirements and defined parameters by regulation [3].

Thus, this study aimed to present methodologies for the extraction and detection of antibiotics in matrices of animal origin by applying liquid chromatography coupled to a mass spectrometer, specifically UHPLC-QTRAP-MS/MS and UHPLC-TOF-MS. According to Regulation 2021/808, this equipment allows us to achieve a deeper knowledge about the occurrence of pharmaceutical contaminants and levels of contamination in food [3].

This work resulted in new advances in the area with validated multi-detection methods for 45 molecules of different classes of antibiotics for fish, meat and milk matrices. Currently, a new method isbeing studied for validation of egg matrix in which it is intended to detect about 60 molecules. Thereby,this work developed food safety tools according to the One Health approach, contributes to the well- being of the population and brings benefits to public health.

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[D&PA 26]

Testing of anthocyanin-based double emulsion colourant systems in yoghurts

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In recent years, the demand for natural products has increased partly due to the growing consumer interest in a healthier lifestyle, which is not compatible with the use of synthetic additives, as they can cause serious health complications when ingested. In this context, one of the most notable groups of water-soluble natural colourants is the anthocyanins group. Their colour depends on the pH conditions and can vary in the range of red, purple, blue and violet [1, 2]. The emulsion systems, specifically double emulsions, have improved performance in protecting anthocyanins in different pH and temperature environments, rendering their colour more stable [3]. In this context, the present work aimed at developing double emulsions incorporating the extract *of Daucus carota* L. as a strategy to improve its colour stability. The primary emulsion comprised water, corn oil, and PGPR as stabilisers, while tween 80/gum Arabic was used as stabilisers of the secondary emulsion. Across the various primary emulsion/water ratios tested using an experimental design, it was concluded that the 50/50 (v/v) ratio containing 8% (w/w) of colourant led to the emulsion showing the best results concerning emulsion stability and colour power. The influence of different temperatures and pHs was then evaluated having in view the incorporation into a yoghurt food matrix. Thus, the emulsion-based colourant system was used to process solid and stirred yoghurts. It was concluded that the system was appropriate for the processing of stirred yoghurt, where the colourant was applied after yoghurt production, with no perceivable colour changes over 15 days. Furthermore, the titratable acidity, pH, and syneresis of the stirred yoghurt incorporated with emulsion remained according to the FDA guidelines during this same evaluation period.

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[D&PA 27]

Solid dispersions as an approach for food colourant systems

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Widespread since 1960, solid dispersions (SDs) have been used as a valuable approach to develop stable and effective drug formulations. The principle is based on the state shift of a hydrophobic active material from crystalline to amorphous via hydrogen bonding, improving the final water solubility and stability [1]. Although it is a widespread technique in the pharmaceutical industry, food area can also benefit from this approach, and some research studies to transpose this technology to overcome limitations of natural compounds, e.g., water solubility, off-flavours mitigation, and natural colouring solutions and under study [2,3].

In this regard, this work proposes employing the SD technique to produce curcumin-based colourant systems. Five natural polymers were included in the formulations, namely k-carrageenan, maltodextrin,Arabic gum, potato starch, pectin, and synthetic polyvinylpyrrolidone. Six formulations with pH control and six formulations without pH control were developed. The SDs characterisation comprised Fourier- transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction, and water solubility determination. The results revealed that the curcumin structure was modified, with a significant reduction of the crystallinity for all systems. Water solubility increased considerably in the SDs compared to untreated curcumin, and the values were higher in most systems developed without pH control. The produced SD particles demonstrated interesting properties to be employed in other foodapplications beyond the direct incorporation as colourants.

Moreover, the particles enabled the development of Pickering emulsions with the potential to replace conventional ones, combining advantages while maintaining the same sensory properties. Another possible application is the incorporation of SDs into polymeric films for food packaging, adding bioactivity to the final product. Overall, this study demonstrated SDs as an excellent option to produce curcumin- based colourants, as well as to be expanding to other relevant applications in the food area.

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[D&PA 28]

Fruit Flours: a sustainable and functional ingredient in the development of vegetarian ham-like products

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The growing popularity of vegetarian alternatives of meat products is an exciting trend in the food industry. Rising interest in these substitutes is driven by health, environmental, and ethical concerns, as well as the emergence of innovative alternatives that closely mimic the texture and taste of animal-based meats [1]. Another important factor is the application of clean label ingredients, that have the potential to increase the appeal of natural-based meat substitutes by reducing their environmental impact, improving product quality, and enhancing health benefits [2]. To achieve this, reusing food by-products that can act as technological ingredients (*e.g.* thickening, emulsifying agents or water retainer agents), while providing health benefits, is a valuable option. The incorporation of fruit flours, as a clean label ingredient, with high amount of dietary fibers can help to modulate the texture to vegetarian ham-like products [4]. Additionally, due to the presence of bond phenolics, fruit flours can also contribute to the development of food products with improved nutritional properties and health benefits.

The main objective of this work is to produce vegetarian ham-like product, incorporating fruit flour into the formulation. Firstly, fruit flour was characterized regarding dietary fibers content and phenolic compounds composition and bioactivity. Several formulations were tested, varying the percentage of fruit flour incorporation (4, 8 and 12 % (w/w)) and testing different fruit flour granulometries (125 μm, 250 μm and 500 μm). The colour and texture of each formulation was determined. Preliminary results showed that higher incorporation of fruit flour resulted on a reduction of the hardness and cohesiveness of the vegetarian ham-like formulations, compared to the control formulation. Higher granulometries changed the texture properties (lower hardness) in all the tested formulations. The application of fruit flour showed promising results in the development of vegetarian ham-like products. Therefore, incorporating these food by-products into a food product allows the addition of multifunctionality, beside contributing to the circular economy.

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[D&PA 29]

Validation of a Peptide Nucleic Acid Fluorescence *in situ* Hybridization for the specific detection of *Salmonella* species in food matrices

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Salmonella is a Gram-negative flagellated rod-shaped bacterium that is one of the most important etiological agents in bacterial foodborne diseases [1,2]. Despite human salmonellosis generally presenting as a self-limiting episode of enterocolitis, the infection can degenerate into chronic and debilitating conditions [2]. To diagnose a *Salmonella* infection, standard cultural methods are routinely used, which implies bacterial identification by biochemical and serological tests, to confirm the suspectcolonies grown on the selective agar [3]. However, this methodology is time-consuming and takes too long to deliver the results [4]. Due to these limitations, more rapid techniques for detection have been developed [5-7]. For that, in this study, we developed a novel Peptide Nucleic Acid Fluorescence *in situ* Hybridization (PNA FISH) method for the specific detection of *Salmonella* spp.

The method was based on a new PNA probe, SalPNA1692, coupled with a novel blocker probe ina 1:1 ratio. The method was optimized for the detection of *Salmonell*a in food samples through an evaluation of several rich and selective enrichment broths. The best outcome was achieved using Buffered Peptone water as a pre-enrichment for 24 h followed by 16 h of selective enrichment in RambaQuick broth. For validation in food samples, fresh ground beef was artificially contaminated with two ranges of inoculum: a low level (0.2–2 CFU/25 g) and a high level (2–10 CFU/25 g). For both levelsof contamination, the confirmed positives were the same comparing the PNA-FISH method and the reference method (ISO 6579-1: 2017). The new PNA-FISH method presented a specificity of 100 % and is a faster time-to-result method, making it a good candidate for routine application in food safety laboratories.

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[D&PA 30]

Impact of grass pea sweet miso on vegan emulsions rheological properties and stability

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Lathyrus sativus, commonly known as grass pea, is a pulse with historical significance in Portugal, particularly in poor and calcareous soils from Centre South. Despite being very nutritious [1], this crop was disregarded over time, due to its content of neurotoxic amino acids related to neurolathyrism. Nevertheless, autochthonous seeds with lower toxicity [2], adequate culinary preparation methods, and the fact that this pulse is not the dominant component of the Portuguese diet, in contrast with what happens in several Asian and African countries [1], has prevented outbreaks in Portugal.

Previous to this work, a grass pea sweet miso was developed to promote this legume's consumption [3], combining the advantages of grass pea, miso, and fermented foods in general. Our goal is to use this innovative fermented paste as a clean label ingredient to develop an innovative vegan emulsion with added value and in line with the most recent food trends.

Five formulations, with 5-15% (w/w) miso paste were tested. Miso emulsions presented distinct physical atributes from the standard, namely higher adhesiveness and viscosity and higher firmness and structure parameters dependent on miso concentration. DSD and backscattering showed that the incorporation of miso did not destabilize the emulsion. SEM results showed that miso emulsions present a typical emulsion structure with some peculiarities related to miso incorporation, specifically bigger and less rounded droplets (also confirmed on DSD). A phenomenon of exudate release was observed and determined on the backscattering assay. This problem was solved by adding 0.1% (w/w) of psyllium husk, a fibre that also improved the nutritional quality of the product.

The incorporation of grass pea sweet miso as a clean-label ingredient was successful, creating new emulsions fit for a consumer eager for innovation aligned with health and sustainability.

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[D&PA 31]

Study of the *koji* mold: the first step towards producing Portuguese *miso*

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Miso is a Japanese fermented food, highly nutritive, digestible and with physiological and functional benefits. Soybeans, koji and salt are the main raw materials for miso production. Koji results from inoculating Aspergillus oryzae in steamed rice and plays a crucial role in miso production, providing enzymes capable of hydrolysing proteins and polysaccharides. Fermentation performed by A. oryzae, yeasts and lactic acid bacteria results in a semi-solid paste – miso – with a strong flavour and the abilityto mask odours while adding umami and depth to various products [1].

With the focus on producing innovative miso using traditional Portuguese protein-rich pulses (lupin, chickpea and cowpea) instead of soybeans, the first step to koji production was to select the A. oryzaestrain, considering fungus' growth and sporulation capacity, and its hydrolytic potential.

Fungal strains were obtained from microorganism collections (AL and AJ) and from commercial koji (ABand AS). After determining the total number of spores and cell viability, the enzymatic activity was evaluated by inoculating fungi in specific media in the absence and in the presence of 3% and 12% NaCl, supplemented with starch (amylolytic activity) and casein (proteolytic activity). Amylolytic, proteolytic and lipolytic activity assays were performed according to previous studies [2-4].

AS and AJ, presented the highest values for amylolytic (0.620 and 0.615 U/mL) and proteolytic activities(12.125 and 7.340 U/mL) in the absence of salt. Their enzymatic performance was also high in the presence of salt. AS presented the highest value for lipolytic activity (1.122 U/mL), as well as a high growth rate.

According to these results, AS strain was selected as the best fungus to produce koji for Portuguese innovative miso, in order to obtain more nutritious and sustainable foods, and support national producers.

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[D&PA 32]

Assessment of a Biochip Immunoassay for Multi-Mycotoxins Screening in Rice and other cereals

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Co-contamination of mycotoxins in foods and feedstuffs has been extensively reported, therefore the interest on protection of animal health and consumer safety has grown [1]. The developing methods of analysis have to be sensitive, affordable and accurate for the control of mycotoxins. Recently, immunoassays have become promising candidates for mycotoxins analysis. This study focusses on a validation of a biochip array technology for multi-mycotoxins screening in rice, and was extended to other cereals, like, oat, barley, rye, and wheat. The Evidence Investigator Myco 7 (RANDOX Food Diagnostic), based in a competitive chemiluminescent immunoassay, was used for the simultaneous semi-quantitative detection of the mycotoxins immunoassays: aflatoxin B1 (AFB1) and aflatoxin G1 (AFG1), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 and HT2 (sum of T2 and HT2), fumonisins (sum of FB1 and FB2) and deoxynivalenol (DON) [2]. A single extraction step with acetonitrile:methanol:water (50:40:10, v/v/v) was used. According to validation results, spiked rice samples showed low false results (5% of false negatives and 5% of false positives in fumonisins (FB1 and FB2), 5% of false negatives in ZEA, OTA, AFB1, T2HT2 and DON), in agreement with EU legislation performance criteria [3]. In the spiked samples of other cereals any false negatives and false positives were found. The validated biochip array technology immunoassay offers significant advantages in the high-throughput cost-effective screening of mycotoxins and enables quick screening of multi-mycotoxins from cereals samples at different levels. This multi-analytical approach also facilitates the screening process because only samples suspected of contamination will be submitted to the confirmatory testing.

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[D&PA 33]

Detection, quantification, and mitigation of mycotoxins in rice

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The occurrence of mycotoxins in the food is linked to the potential contamination of crops, primarily cereals, resulting in an unavoidable increase in human exposure. Rice, the world's second most consumed cereal, is a significant source of potential mycotoxin contamination. Since the prediction scenario of mycotoxins contamination is to increase with climate change and global warming, the key to minimizing their occurrence must be based on prevention and control.
The European Commission's Rapid Alert System for Food and Feed (RASFF) portal and scientific publications reported an increasing number of notifications and the occurrence of mycotoxins at levels above the legislated limits [1]. The reported data justifies the objective of this study which purpose is to compile the most relevant studies and review the main methods used on the detection and quantification of mycotoxins in rice. Furthermore, the toxic effects of mycotoxins contamination were reviewed, as were the techniques used to mitigate that contamination.
The predominant mycotoxins detected in rice grain (brown, white) and rice flours are aflatoxins and ochratoxin A and this data highlights the importance of adopting safe storage practices that prevent the growth of fungi from the *Aspergilus* genus [2]. Immunoaffinity columns (IAC) and QuECHERS are the elected for extraction and purification methods, while HPLC-MS/MS is the most widely used quantification method [3]. Given the continuous evolution of methods, it is expected that these techniques will be replaced by high resolution mass spectrometers such as Orbitrap and Time of Flight (ToF).
More research is needed to determine the real exposure to these contaminants, as well as the consequences and potential synergistic effects due to the co-occurrence of mycotoxins. Therefore, it would be of great importance to carry out more studies in order to evaluate the impact of climate change on rice contamination by mycotoxins.

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[D&PA 34]

Lithium occurrence in food produced in the surroundings of the C-57 mine

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The interest in lithium (Li) has grown over the last decades, mainly due to its application in electronic products and electric vehicles (2020, Kaunda). Li is not considered an essential nutrient for vital functions, and at high levels could be toxic to humans (2021, Bolan).
Portugal is a country with active and prospective Li mining concessions. This pioneering study aims to evaluate the Li contents in cabbages and irrigation water in rural areas near the C-57 Li pegmatite mine in Gonçalo (Guarda district, Portugal). The distance between the sampling locations and the mine classified them as near (up to 1,5 km, L1) or far (between 2 and 8,5 km, L2) to the mine.
Twelve subsistence farms constituted the sampling plan in both locations. Three cabbages were collected in each farm (n=72) and analysed as a laboratory pool (n=24); water samples were also collected at each location. Li amounts in the selected samples were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and cabbage analysis was preceded by microwave-closed vessel acid digestion. Analytical procedures were conducted following the quality assurance requirements described in the ISO/IEC 17025:2017 standard. The Li concentration in each location was expressed, as the average of three replicates, in µg/kg of fresh weight for cabbages and µg/l for water. The results from L1 are, on average, higher than those found in L2. On cabbage samples, the Li ranged from 44.4 ± 0.7 µg/kg to 1626 ± 32 µg/kg µg/kg in L1 and from 33 ± 1 µg/kg to 509 ± 19 µg/kg in L2; on irrigation water the Li levels ranged from 6.1 ± 0.1 µg/l to 36.6 ± 0.4 µg/l in L1 and from 2.40 ± 0.04 µg/l to 16.7 ± 0.4 µg/l in L2.
The results show the influence of the geogenic lithium resource proximity.

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[D&PA 35]

Ultrasounds: a new approach to seaweeds fermentation process

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Macroalgae have a huge potential as a substitute for animal products due to its rich nutritional content in fatty acids, proteins, vitamins, fibers, minerals, and bioactive compounds[1]. With the rising interest in plant-based diets, new ranges of opportunities arise for the development of innovative products. ***FermentedVegAlgae*** project aims to develop fermented products from macroalgae and vegetables, offering, innovative, healthy and sustainable food products that meet consumer demands.

According to Uchida[2], one of the difficulties in fermenting macroalgae is the high content of non-fermentable fibers, such as cellulose and other polymers, which decreases nutrients availability for fermentation. In this work, ultrasound pre-treatment was applied for cell disruption and microbial inactivation of indigenous microbiota of two Portuguese indigenous coastal macroalgae - *Alaria esculenta* (brown) and *Palmaria palmata* (red), without compromising their organoleptic characteristics. The pre-treated macroalgae were used to produce two sauerkraut-like fermented products. Fermentation assays were performed using a consortium of lactic acid bacteria (LAB) and yeast in the presence of 0.5% added salt, until pH stabilization.

Pulsed and continuous ultrasounds treatments were tested. The best results were obtained at 25 kHz (acoustic energy density 0.95 W/mL), between 30-70 W (100% amplitude) and 30-40 ºC (depending on the algae matrix and polysaccharide content). For *Palmaria palmata* and *Alaria esculenta*, continuous treatments of 3 and 4 minutes were applied, respectively.

The release of cellular content was clearly observed under the microscope. Pre-treated *P.palmata* and*A.esculenta* fermentation reduced pH bellow 4.50 in the first 72 hours, stabilizing at 3.72 and 4.06 after 7 days, respectively. These values are in accordance with the production of lactic and acetic acids by LAB. For *P.palmata,* ºBrix evolved in accordance to pH, decreasing to 7.53 at 4th day, while *A.esculenta* maintains 2.5 °Brix for the 7 days of fermentation. This approach improved seaweed fermentation, reducing time and increasing product safety.

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[D&PA 36]

3D printing snacks incorporating meal from *Tenebrio molitor* and Alphitobius diaperinus

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The expected increase of the world's population is 9 billion in 2030 and 10 billion in 2050 (UN, 2022). As developing on the current production system is not a viable option (APA, 2021) alternatives must be developed. This work aims to contribute to the progress on novel-food. Assays were carried out with two edible species of insects: Tenebrio molitor and Alphitobius diaperinus, to analyse their nutritional properties. With 3D printing technology it is intended to present, to the Western consumer, a nutritious and tasty alternative with an appellative design.

An original formulation for the printing of duck footings with micro algae (Letras et al., 2021) was adapted to incorporate the edible insects. From the formulation previously established for snacks, the recipes were tested replacing 10% of the flour: (a) with meal of T. molitor; (b) with meal of A. diaperinus (Figure 1); and (c) with 5% T. molitor + 5% flour A. diaperinus. Stress sweep and frequency sweep tests were performed on the doughs, to understand the degree of internal structure to better suit these materials to the 3D printing task. The adjustment of the water content of the doughs was performed using the Micro-doughLab equipment.

The nutritional profile of the developed snacks was assessed, revealing a significant amount of protein, enough to claim the snacks as “source of protein” (EC No 1924/2006), as well as an increased mineral profile, when compared to the control snack. The antioxidant profile and total phenolic compounds were equally assessed. The high antioxidant capacity of the flour of both insects was confirmed. A sensory analysis test was performed, comparing the control snack to 3 other samples containing 10%
T. molitor, 10% A. diaperinus and 5% + 5% of T. molitor and A. diaperinus respectively, resulting on a preference for the A. diaperinus and for the combination of the two insects. Further study of the properties of edible insects must be conducted to better understand the added value of these products to the current market of alternative sources of nutrients.

Figure 1. Successfully 3D-printed A10% snacks

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[D&PA 37]

Development of a Clean Label Meat-free Alternative to Deli ham

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To minimize environmental impact, reduce the development of health-related problems and make food production sustainable, the number of people who choose to completely exclude meat from their diets has increased[1]. Therefore, there is a need to develop meat analogues or meat alternatives. The concept of "meat analogues" refers to high-protein food products, without meat, but providing a similar experience: are conveniently used in a similar occasion; have similar texture, appearance, nutritional value and taste, to the replaced meat products. However, meat analogues are generally highly processed products[1]. Thus, the objective of this work was to develop a clean label Meat-free Alternative to Deli ham (MAD) without any food additives, for a Portuguese company.

Since the main category of food additives used in MADs are thickening agents, 7 natural hydrocolloids were tested: pregelatinized banana starch, psyllium, cassava flour, inulin, fructo-oligosaccharides, rice flour, and quinoa flour. To evaluate and compare the prototypes, colour, pH, moisture, texture and rheology parameters were measured. These results were evaluated by comparing the prototypes to the original formulation (with additives) and to a targeted MAD. The most promising hydrocolloid was psyllium as it forms a homogenous, strong, non-friable and elastic gel, with no significant differences ($p > 0.05$) to the target product in the resulting values of firmness and moisture. Psyllium addition decreased the water activity value (a_w), due to its water holding capacity. Regarding the rheology behaviour, both mechanical spectra revealed the same pattern showing a structured gel behaviour with G' over G'' and a slight frequency dependence. However, the psyllium prototype showed significant ($p < 0.05$) higher values of both G' at 1Hz and G_0^N, revealing a stronger gel structure.

Research on this area is essential to a clean and sustainable food production and to offer alternatives to meat without compromising the use of natural ingredients.

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[D&PA 38]

New insights into polyphenols-proteins complexes as natural emulsifiers in mayonnaise models

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Phenolic compounds (PCs) are bioactive compounds which can be described as promising tools to reduce the use of synthetic additives[1]. Besides the health-promoting effects[2], PCs have been described as able to modulate the main organoleptic characteristics of plant-derived foods and beverages[3,4] while they can be also used as antioxidant or antimicrobial agents[5]. Moreover, their natural ability to bind to proteins can bring a new added value into the use of PC as promotors of emulsification.

In this study, we employed several PCs as promotors of emulsification of a yeast protein extract (YPE)[6] – based mayonnaise with different fat contents. The goal was to use a combination of PCs and a novel YPE as emulsifiers for vegan mayonnaise. Several tests were carried out on a small and medium scale to better mimic the conditions used in the food industry.

Overall, the alternative emulsions obtained were similar to egg-based traditional mayonnaise. The addition of PCs or PC-rich extracts improved the rheology and texture properties of our YPE-based full fat mayonnaise. Onion powder presented similar rheological properties, while blueberry extract and powder, grape seed extract, and wine extract presented superior results compared to commercial control. Despite the obvious colour difference that some of the PC-rich extracts provided to the final product, there is a significant potential for the complete replacement of egg protein for the stabilization of mayonnaise.

Similar formulations were made on an industrial scale with low-fat mayonnaise, and preliminary results are very positive, with a significant reduction in fat being possible without compromising the final product organoleptic characteristics. The physic-chemical characterization of the most promising low-fat new products will be performed.

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[D&PA 39]

Determination of cobalamin (vitamin B12) in microalgae biomass by HPLC-UV

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Within the transition from animal into plant-based food, our ability to rethink our food consumption profile is more important than ever, to ensure healthy feeding of a fast-growing population. This new paradigm originated the concept of functional products and induced a higher demand for them. The list ingredients includes omega-3, polyphenols, proteins and vitamins. Several prominent companies have become involved with the production of redesigned products with nutritional allegations. Therefore, analysis of fortified foods is required, to confirm allegations and promote clear and trustful labelling.

The current project is focused on the determination of cobalamin, a water-soluble essential vitamin that is found naturally in animal-based foods, but also available as a dietary supplement. Vegetarians are likely to develop deficiency on it, since it is only biosynthesized by certain protozoa, and therefore accumulated along the food chain [1]. Four types of *Chlorella vulgaris (C. vulgaris)* strains were assessed. Extraction was carried with sodium acetate buffer (at pH = 4 and 100 °C for 30 min), in the presence of sodium cyanide and enzyme hydrolysis (α-amylase, 40 °C), followed by a purification step with immunoaffinity columns [Easi Extract® Vitamin B12 (LGE)]. Cobalamin was monitored by UV detection at 361 nm after separation on TCC-3000SD column with isocratic elution, with acetonitrile and trifluoroacetic acid 0.025% (15:85) as mobile phase at 0.25 mL/min flow rate [2].

The autotrophic *C. vulgaris* (designated as organic) displayed the highest levels of total cobalamin, around 990 μg/100 g. Smooth (hetetrophic-light green) and Honey (hetetrophic-yellow) *Chlorella*, yielded values around 270-300 μg/100 g. The White variant showed the lowest levels, (around 250 μg/100 g). Actually, 400 mg of organic *C. vulgaris* would be enough to comply with the total RDA.

Our data provides new insights on the possibility of improving vegan products using microalgae as a sustainable ingredient.

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[D&PA 40]

Sustainable and Nutritional Potential of Healthy Protein-Rich Backed Snacks Using Organic Pea (*Pisum sativum* L.) Flour

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Recently, health and nutritional benefits of pulses and their by-products have gained significant attention from researchers and consumers, as they represent one of the largest plant-based meat alternatives (PBMAs). Pulses have a positive impact on human health, contributing to reduce the risk of cancer and coronary hearth diseases or diabetes (1, 2). Among pulses, peas (*Pisum sativum* L.) are an important source of high-quality proteins, dietary fibres, resistant starch, vitamins and minerals and highly promoting antioxidants (3). Moreover, environmental studies demonstrated that plant-based products generate a significantly lower GHG emissions than dairy production (4), thus representing a valid and sustainable substitute for animal-based products.
In this work, organic peas were studied to assess the impact of different growing locations (during the 2021 and 2022 cultivation cycles) on the nutritional profile of the grain. In addition, the incorporation of organic pea flour on wheat savoury snacks (crackers) was studied. Appearance, physical properties (texture) and biochemical composition of the crackers was investigated to obtain a final plant-based product with high-quality nutritional content.
Peas (*Pisum sativum* L.) were cultivated under low-input agricultural systems in two different Italian locations for each year of cultivation: Loiano (Bologna, Italy) and Ozzano dell'Emilia (Bologna, Italy) and Loiano and Cadriano (Bologna, Italy) for the years 2021 and 2022, respectively. At the end of the fruit development (BBCH-79), harvested grains were dried and milled as flour to be evaluated in terms of proteins, ash, minerals, and B6-vitamin (pyridoxine) content, in order to determine the nutritional profile of the samples. Previous nutritional analysis on the pea flours showed a significative difference between the different growing locations, hence it was chosen to prepare crackers enriched with 6% (w/w) of pea flour from the different sites, as described by Mota et al., 2021 (5), to perform rheological, texture and sensory studies (6).

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[D&PA 41]

Effect of the microalgae incorporation on the rheological, textural and nutritional properties of a texture modified vegetable puree designed for people with dysphagia

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The ingestion of texture-modified foods is the most used strategy to avoid aspiration, choking or malnutrition in people with dysphagia. These foods must be soft, safe and easily swallowed, but also nutritive and sensory attractive (Gallego et al., 2022).
Since microalgae are commonly used as food supplements because of their high-protein content, minerals, essential fatty acids and vitamins, their addition to dysphagia foods can contribute to obtain high nutritional value dishes (Schüler et al., 2020).
This work aimed to develop innovative vegetable purees using microalgae. The control formulation containing 10% zucchini, 10% carrots, 54.88% water, 21.82% lentil protein concentrate (LPC), 2.5% extra virgin olive oil and 0.8% xanthan gum (XG) was firstly developed. Then three formulations with 3% of smooth chlorella (SC), honey chlorella (HC) or white chlorella (WC) were prepared by replacing the corresponding 3% of LPC.
Rheological properties (SAOS), textural (firmness), color (CIEL*a*b*) and nutritional parameters (minerals) were analysed.
A slight increase was observed for elastic modulus (G') after adding SC and HC when compared to the control one. Meanwhile, for the WC, a slight decrease was observed. Tan δ ranged from 0.185 to 0.514 for all the samples, indicating a weak gel structure, considered a rheological criterion for safe-swallow foods for dysphagia (Ishihara et al., 2011).
The firmness of the samples decreased as follows: Control>SC>HC>WC. Regarding color parameters WC samples exhibited similar values to the control ones followed by samples containing HC and SC. The total mineral content of the puree improved with the microalgae addition making these a good nutritional value dish.
Results indicated that the microalgae incorporation caused small variations on the values of every parameter (rheological, textural and color properties) also improving the total mineral content. All formulations may be suitable for people with dysphagia.

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[D&PA 42]

Detecting Enterotoxigenic *Escherichia coli* in animal production:method development and validation

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Swine enteric colibacillosis is a disease characterized by an intestinal infection caused by the colonization of enterotoxigenic Escherichia coli (ETEC). This infection mostly causes illness or death in neonatal and weaned pigs making it responsible for significant economic losses worldwide [1,2]. Bacterial fimbriae (F4/F5/F6/F18) allow the adhesion of the bacteria to epithelial cells, and when both the immunological systems and the gut microbiota are poorly developed, ETEC colonizes and producesone or more enterotoxins (LT/STa/STb) that can have local and systemic effects [3,4]. Therefore, it is ofprime importance to monitor and characterize ETEC in the swine industry to develop mitigation strategies.

In this study, we aimed to isolate and characterize ETEC strains from faecal porcine swabs. As such, itwas developed a methodology to detect the presence of ETEC and their major virulence factors (toxins/fimbriae). This procedure was divided into two phases, firstly the collected swabs were enriched and then then screened for the presence of genetic determinants of toxins (ST/LT/stx2) by real-time PCR (qPCR). Secondly, the positive enrichments were plated in Tryptone Bile X-glucuronide (TBX) agarand incubated at 37 ºC for 24 hours. Fifty characteristic *E. coli* colonies were then extensively screened for the presence of toxins (Sta/STb/LT/stx2e) and fimbriae (F4/F5/F6/F18/F41) by multiplex-PCR.

The development of both qPCR/multiplex-PCR methods, as well as the optimization of the enrichment step, was done using ETEC controls harbouring the above-mentioned toxins/fimbriae. Nonselective andselective (with novobiocin) enrichment in TSB was performed by using ETEC inoculated faeces samples; with the 24h-selective enrichment providing higher ETEC recovery rates. Optimized qPCR conditions for toxins detection were as follow: 95 ºC for polymerase activation/denaturation, 60 ºC for annealing/extension during 40 cycles, and an internal control (pUC19 DNA) was used in each reaction.Multiplex-PCR was optimized through the conditions, 95 ºC initial denaturation and 35 cycles of 94 ºC denaturation, 60 ºC for annealing and 72 ºC for extension/final extension.

In sum, this methodology has the potential to be adopted as a routine technique for the rapid detectionof ETEC strains in livestock.

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[D&PA 43]

Aptamers for blocking enterotoxigenic *Escherichia coli*

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The most common bacterial pathogen causing enteric infections in swine is enterotoxigenic Escherichiacoli (ETEC). ETEC-associated diseases, lead to acute diarrhoea and eventual death of the animal, resulting in significant costs to the swine industry [1]. The toxins/fimbriae produced are essential for theirpathogenicity. Fimbriae are responsible for the first adhesion of ETEC to the intestinal epithelial cells, giving rise to the onset of infection. In particular, the F4 type (K88) fimbriae are frequently attributed to neonatal infections and most post-weaning diarrhoeal infections [2]. These diseases are traditionally prevented or treated with antibiotics, but antibiotics use is becoming highly restricted due to the emergence of resistant bacteria and its implications to human health [3]. Thus, the development of aptamers (small single-stranded oligonucleotides capable of binding to target molecules with great affinity and specificity [4]) to target the F4-type fimbriae and block the initial ETEC adhesion is an alternative.

The present study focuses on the binding and affinity testing of a pre-selected aptamer for ETEC fimbriaagainst the F4-ETEC strain and other bacterial strains (F5, F41-ETEC, F6-ETEC, F18-ETEC, Staphylococcus aureus ATCC 25929, Klebsiella pneumonia ATCC 43216, Escherichia coli K12) by quantitative PCR. Then, we tested the aptamer toxicity in Galleria mellonella by inoculation of different concentrations (1 µM, 10 µM, 20 µM) in comparison to a control solution (PBS). Relatively to the specificity and affinity of the aptamer, preliminary results showed a good affinity to ETEC-fimbria; however, somecross-reactions with other bacterial species with similar fimbriae to those of ETEC was observed. Aptamer did not demonstrate any toxicity in Galleria mellonella after 96h independently of the concentrations of the inoculated aptamer. Further research will be carried on to assess the survival of Galleria mellonella pre-infected with ETEC and subject to aptamer treatment.

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[D&PA 44]

Antimicrobial activity of an edible film incorporated with oregano essential oil

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Microbial food contamination has been the target of increasing attention. To avoid or minimize it, the use of appropriate packaging systems, which can act as a barrier and protect the food against spoiling microorganisms has been evaluated[1]. Edible films can be a way to overcome this problem due to their functional properties that allow them to extend food's shelf-life[1].

Essential oils are Generally Recognized as Safe substances and have been used in food products as natural flavoring agents. Due to their high concentration in phenolic compounds, they have antimicrobial activity against a wide range of microorganisms[2]. Oregano essential oil, in particular, possesses a strong antimicrobial activity against Gram-positive and Gram-negative bacteria[3]. However, high concentrations of oil are required for it to exert its activity, which may exceed organoleptic acceptable levels[4]. The incorporation of essential oils onto films is able to circumvent these problems.

So, the main objective of the present work was to develop alginate films with two concentrations of oregano essential oil (2% and 3%). The antimicrobial activity of both formulations was tested by the viable cell method against bacterial species *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salmonella enterica* serovar Enteritidis, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus.*

The results showed that both films were able to inhibit the growth of all studied bacteria. However, it was necessary more time to completely inhibit the growth of all studied bacteria when exposed to the film with the lower concentration of essential oil (for example, *E. coli*'s growth was completely inhibited after 2h of incubation when exposed to the film with 3% of oil but, when exposed to the film with 2% oil, the same bacteria required 4h to be completely inhibited).

So, the developed edible films are potential natural solutions to prevent microbial growth in food products and extend shelf-life.

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[D&PA 45]

Development of an aptamer-based biosensor for the detection of food toxins

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According to the last EFSA/ECDC report, bacterial toxins are the second leading causative agent (17.0%) of foodborne outbreaks in Europe [1]. Aptasensors, as biosensors that use aptamers are called,are seen as a simple, rapid and cost-effective assay format with high suitability for point-of-care testing,allowing a sensitive and mostly qualitative detection of analytes [2].

In this work, DNA aptamers previously selected by our group (Apt1, Apt2, Apt3, Apt4 and Apt5) for staphylococcal enterotoxin A (SEA), one of the most reported bacterial toxins, were applied asrecognition molecules in a lateral flow assay. For this, lateral flow strips consisting of a sample pad withglass fibre, test zone with nitrocellulose membrane and absorbent pad with cellulose membrane were assembled. Gold nanoparticles (AuNPs) covalently attached with Apt5 were synthesized. Biotinylated aptamers (140 pmol of Apt1, Apt2, Apt3 and Apt4) were immobilized in the test zone using streptavidinas an anchor. A DNA probe (140 pmol) complementary to Apt5 was also immobilized as a test control.Then, SEA solutions (0.3 ng/µL) as well as negative samples were prepared and incubated with Apt5- AuNPs (OD3) in binding buffer for 10 min. Different assay combinations (Apt5-AuNPs +Apt1/Apt2/Apt3/Apt4) were tested. The samples (60 µL) were applied to the sample pad, allowing the solution to flow on the strip until the test lines were visualized by the accumulation of AuNPs.

SEA samples were positively detected, with the combination of Apt5-AuNPs with Apt3, providing the best result, followed by Apt4, Apt2 and Apt1. Negative controls were validated by the control line. Further tests to determine the detection limit and improve the noise ratio are being carried out.

These results show that aptasensors can be a simple and rapid alternative for the detection of SEA. Furthermore, this format assay can be easily adapted to any food toxin.

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[D&PA 46]

Acylated and polyacylated anthocyanins as a pallet of natural colors

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Anthocyanins are colored pigments belonging to the phenolic group that comprises a diverse group of intensely colored pigments responsible for the colors, red, purple, and blue, are in fruits and vegetables. They are water-soluble, which facilitates their incorporation into aqueous food systems. Besides the color attributes, interest in anthocyanins has intensified due to their health benefits as antioxidant, anti-inflammatory, anti-mutagenic, anticancer, and neuroprotective effects. However, these compounds are strongly affected by different factors such as pH, temperature, and light that restrict their use as natural colorants for example in food systems.[1] Anthocyanins whose glycosyl moieties are acylated by hydroxycinnamic acid residues (e.g. coumaric, ferulic, sinapic acids) are known to exhibit more stable colors than nonacylated precursors.[2] This improvement in the stabilization is achieved through π-stacking interactions between the hydroxycinnamic acid and anthocyanidin chromophore, which can involve individual anthocyanin molecules (intramolecular copigmentation) or noncovalent dimers (self-association) and possibly higher oligomers.[3]

This work proposes the use of a green extraction methodology to obtain acylated and polyacylated anthocyanins extracts from two different edible sources, namely the red cabbage (RC) and the blue butterfly pea flowers (BPF) using hot water. Extracts' chemical composition in terms of sugars, lipids, proteins, anthocyanins, and other phenolic compounds was determined using different methodologies. The color stability of an aqueous solution of each extract was also evaluated over time at different pH values (Figure 1). Knowing that color plays a key role in establishing consumer acceptability of food and also that there is a current need for the substitution of synthetic colorants by natural ones, acylated anthocyanins can be the key to obtaining a pallet of natural colors with biological properties for the creation of innovative functional foods.

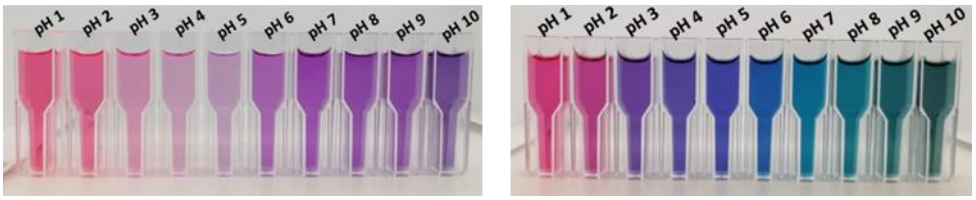

Figure 1: Color expression of acylated anthocyanins at different pH values in extracts of red cabbage (left) and blue butterfly pea flowers (right).

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[D&PA 47]

iLabel: Intelligent colorimetric labels for fish freshness monitoring in real-time

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Nowadays, both food safety and food waste are issues of increasing concern for society, and innovative food packaging systems have been explored to help tackle these problems [1]. Behind the technologies applied in intelligent packaging, colorimetric indicators are one of the most studied in research since they provide real-time information about packaged food [2].

In the present work, a colorimetric pyranoflavylium-based pH-indicator was developed and studied as an intelligent label for food freshness monitoring. Pyranoflavylium pigment at 0.2% (w/w) and glycerol at 30% (w/w) were immobilized in a cellulose acetate matrix, and a thin film was obtained by the solvent casting method. The pH-responsive properties of the film were evaluated in solutions at different pH values (pH 4 to 8). Film photostability and pigment migration over time were assessed. The label's performance was evaluated using real samples (fish and meat) at controlled conditions (3 ºC and modified atmosphere packaging).

The indicator film showed an excellent color change in solutions at different pH values. In terms of pigment migration and photostability, the label showed to be suitable for application in fresh meat and fish packaging. The best performance was observed for the freshness monitoring of fish samples. The indicator demonstrated high efficacy in detecting spoilage of Atlantic horse mackerel (*Trachurus trachurus*). After 5 days of storage, freshness parameters exceeded the limit defined by EU regulations, indicating that the fish was unfit for consumption. Simultaneously, the yellow label started to turn purple (Figure 1), effectively indicating the beginning of the spoilage process.

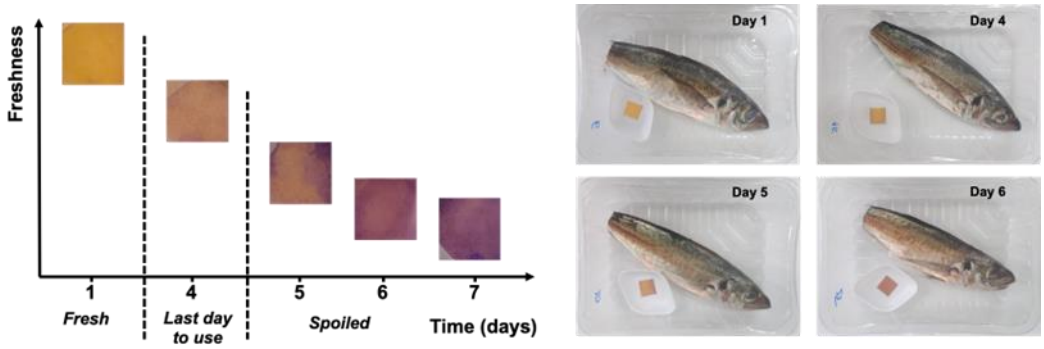

Figure 1. Color variation from fresh to spoiled state of *Trachurus trachurus* sample.

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[D&PA 48]

A comprehensive validation of a liquid chromatography–tandem mass spectrometry method for the determination of chloramphenicol in urine

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Chloramphenicol (CAP) is a broad-spectrum antibiotic generated by *Streptomyces venezuelae*, a Gram-positive bacterium found in soil under particular conditions. The substance was discovered in 1947 and was shown to have antibacterial activity, particularly against Gram-negative bacteria that cause illness in humans [1].

It is commonly employed in animal production because of its antibacterial and pharmacokinetic effects, both in the treatment of sick animals and as a growth promoter. The use of chloramphenicol in animals for food production is not approved in the European Union due to their hazardous effects in Human Health and association with bacterial resistance. However, an RPA (Reference Points for Action) of 0.15 g/kg has been created in order to unify analytical results across EU countries.

Any biological fluid, including urine, can be screened for illegal substances. The fundamental goal of CAP detection is to understand its metabolic process, which is characterized as the conjugation of phase II with glucuronic acid, that results in the formation of chloramphenicol glucuronide (CAPG), which is excreted in the urine [2].

The current approach describes a technique for determining CAP in urine in several animal species using ultra-high-performance liquid chromatography-MS/MS (UHPLC-MS/MS).
The analytical process consists of three main steps: enzymatic hydrolysis with β-glucuronidase (convert CAPG to CAP); solid phase purification of urine extracts and determination by UHPLC-MS/MS.

A robust confirmation methodology for chloramphenicol residues in urine [3] that fulfilled European Commission Decision 2002/657/EC [4] requirements was validated. The lower CCα value (0.012 µg/L), matrix effect conformity (<15%), and precision (RSDr=5.48%; RSDwR=6.70%) demonstrated the feasibility of using this approach in real matrices, ensuring the safety of the presence of chloramphenicol residues in animals intended for human consumption.

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[D&PA 49]

Phytochemical evaluation of cowpea immature pods and grains: a ready-to-eat purée for elderly people

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Cowpea (*Vigna unguiculata* (L.) Walp.) is a nutritionally important crop with a valuable source of health benefits components such as soluble and insoluble dietary fiber, resistant starch, phenolic compounds, and minerals, such as potassium, magnesium and selenium, and B-complex vitamins.[1,2] Cowpea consumption has protective effects in several metabolic and cardiovascular diseases.[1,2]

Previous works had shown that cowpea immature pods exhibited higher phenolic composition and antioxidant activity as compared to immature and dry seeds.[3] This work aims to develop a cowpea immature pod ready-to-eat purée for elderly to promote the maintenance of their muscle mass and synthesis of neurotransmitters implicated in depression disorder and sleep quality. In a preliminary approach, this study intends to assess the phenolic content, antioxidant capacity, through ABTS•$^{+}$, DPPH• and FRAP assays, nutritional composition, and essential and non-essential amino acids by HPLC-FLD of the cowpea at two different growth stages: immature pods and green seeds.

Immature pods showed a significantly higher content of total phenols (11.73 ± 0.43 mg AG/g dry weight), *ortho*-diphenols (13.18 ± 1.26 mg GA/g dw), and flavonoids (6.04 ± 0.51 mg CAT/g dw) as compared to the green seeds. *The higher antioxidant capacity* was also displayed by the pods (ABTS•$^{+}$: 0.05 ± 0.00 mmol Trolox/g dw, DPPH•: 0.04 ± 0.00 mmol Trolox/g dw, FRAP: 0.04 ± 0.00 mol Trolox/g dw) in contrast with the green seeds. Immature pods demonstrated *lowest* crude fat content (1.74 ± 0.03%) and highest content of crude protein (27.48 ± 1.05%) and of insoluble dietary fibre (35.63 ± 0.53%).

To our knowledge, we present the first study concerning the nutritional composition of cowpea immature pod, suggesting that it have remarkable potential to be included in the development of a new functional food product for the elderly, allowing farmers to make their business more profitable and diversified.

Keywords: cowpea immature pods, phenolic content, antioxidant capacity, nutritional composition, high-performance liquid chromatography coupled to fluorescence detection.

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[D&PA 50]

Survival of *Listeria monocytogenes* adhered to inox surface to starving and drying

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In food manufacturing ensure food safety becomes a real challenge when Listeria monocytogenes is present in its environment. This micro-organism is one of the pathogens with higher mortality when causing foodborne infections [1]. This ubiquitous pathogen can persist in food processing environments for long periods (months or years), resisting to different stress factors, for this reason some strains are designated as persistent [2]. Within the group of persistent cells there is evidence for the existence of at least two phenotypic subpopulations, persisters and viable but non-culturable (VBNC). Both cells have the ability to resist antibiotic exposure by entering into a dormant state. These two subpopulations can be distinguished, when removing the antibiotic persisters growth while the VBNC do not have an immediate growth, which may take months or years. [3]. Thus, this work aimed to induce the dormant state in bacteria using drying and starving as stress factors. For this, stainless-steel coupon surfaces were immersed in 35 mL of inoculated Brain heart infusion and incubated with agitation at 25±3 ºC for 24 h. At the end of the 24h, the liquid was removed, and surfaces were washed 4 times with 35 mL of Ringer's solution. Finally, the surfaces were left to dried at 25±3 ºC. From 24h of drying until 12 days counting of L. monocytogenes was performed in ALOA. For this purpose, 35 mL of Ringer's solution was added to the flask containing the surface and then was submitted to stirring and sonication. Results showed a slight decrease in the number of the micro-organism, approximately 1.5 log CFU/mL after 12 days.

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[D&PA 51]

Could grape pomace be considered a good raw material for the muffin's enrichment?

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Nowadays, the use of bioactive compounds present in agro-food by-products as a source of functional andantioxidant ingredients for the development of new products has been increasing. In fact, the agro-industries activity produces massive quantities of by-products, which represent serious environmental and disposal problems, such as for wineries. On the other hand, these residues have been described as a natural source of polyphenols which are mainly responsible for several biological properties[1]. Grape pomace is considered a major solid waste from the wine sector and is composed by skins, seeds,and residual stems after the grapes are pressed and the fermentation processes [2].
Since pomace has high levels of polyphenols, there are studies that incorporate this by-product into foods [3].
In this context, the aim of this study was to evaluate the effects of the incorporation of pomace from theDouro region in the formulation of muffins on their phenolic composition, antioxidant capacity, sensorialcharacteristics, and color parameters. A control muffin was also performed (without pomace). Afterwards,the content of total phenols, flavonoids, ortho-diphenols, and the antioxidant capacity by three different methodologies (DPPH, FRAP, and ABTS) were determined by spectrophotometric methods [4]. The color$L*$ (lightness), $a*$ (redness), and $b*$ (yellowness) of the muffins were also determined using a colorimeter. With this study, it was possible to conclude that the incorporation of this by-product increased the phenoliccomposition and the antioxidant capacity of muffins and originated significant differences in the color parameters compared with the control muffin. Furthermore, the 20% red grape pomace muffin was the onewith the highest acceptability. Regarding the consumer's purchase intention, the red pomace muffins werethe first consumer's choice, followed by the control muffin.
We can conclude that red grape pomace can be an excellent raw material for the enrichment of muffins.

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[D&PA 52]

The analytical challenges of cannabinoids in hemp food products

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Cannabis sativa L. is an herbaceous plant, rich in bioactive compounds, that is widely cultivated throughout most of the world. In general, "medical cannabis" refers to cannabis products used for medical reasons, whether they contain the toxic psychoactive Delta-9-tetrahydrocannabinol (THC) or the non-psychoactive cannabidiol (CBD) [1].
Industrial hemp is the label given to cannabis products used in manufacturing. The legalization of hemp and its rising popularity led to the development and commercialization of hemp-derived foods and phytotherapeutic products that contain C. sativa plant seeds, extracts, oils, or merely its chemical constituents in an isolated form, such as CBD or other cannabinoids. In 2022 Commission Regulation (EU) 2022/1393 [2] established maximum levels of THC in hemp seeds and products derived therefrom. Therefore, analytical techniques are crucial to determine and quantify phytocannabinoids, to confirm if the products are psychotropic-free, impurities-free and if the THC levels are in compliance with the maximum permitted levels.
We present current, emerging, and trending analytical methods used to determine cannabinoids in hemp and hemp-derived food products and an overview of the applicable legislation. Currently, there aren't enough studies that allow us to evaluate the effectiveness and accuracy of each analytical methodology to determine cannabinoids in hemp and in hemp-derived products However, according to the literature, liquid chromatography-based methods are the methods of choice, especially Ultra High-Performance Liquid Chromatography (UHPLC).
Several research papers document the use of liquid chromatography coupled with high-resolution mass spectrometer detectors. However, despite their advantages, these types of equipment are expensive and require specialist maintenance. We believe that the number of hemp-derived functional foods will increase given their potential benefits. However, it is important to continue to monitor the presence of hazardous cannabinoid levels and update legislation according to the newly discovered scientific data. Future research should address the formation of undesirable metabolites obtained from cannabinoid degradation.

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[D&PA 53]

Methods for the determination of multi- mycotoxins in peanuts: current and future trends

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Mycotoxins are toxic secondary metabolites, produced naturally by certain fungi species. Mycotoxin-producing fungi can be found in a variety of foods, including peanuts. Aflatoxins are a prevalent and highly toxic class of mycotoxins produced by Fungi species from the genus *Aspergillus* [1].

During storage and transportation, peanuts are susceptible to aflatoxins contamination and represent a serious threat to human health [2]. Commission Regulation (EC) No 1881/2006 sets maximum levels for aflatoxins (AFB1, AFB2, AFG1, AFG2) in peanuts. However, in 2022 according to the Rapid Alert System for Food and Feed, 41 out of 42 notifications regarding the presence of mycotoxins in peanuts were classified as a serious risk. Therefore, monitoring the presence of mycotoxins in peanuts is crucial to prevent health hazards. There are documented in the literature several methods for the determination of single mycotoxins. However, a vast number of research papers document the co-occurrence of several mycotoxins (such as AFB1, AFB2, AFG1, and AFG2) in peanuts.

We reviewed the mycotoxin multi-analytical methods developed in the last ten years for the simultaneous quantitative determination of regulated and unregulated mycotoxins in peanuts. We verified that HPLC-MS/MS was the most widely used multi-mycotoxins determination method and solid-liquid extraction was the extraction method of choice.

These multi-analytical methods have become more sensitive, accurate, and precise throughout the years. Some of the most promising multi-analytical systems for the determination of mycotoxins in peanuts include IMSPE-UHPLC-MS/MS, Ultra-fast RP-HPLC-FD-DAD, and MagQBD-ICA strip. Furthermore, there is a tendency to avoid clean-up procedures, and add other methodologies (such as ISPME or a combination of QuEChERS and double extraction) to enhance the performance of the methods [3–5].

We anticipate that the development and optimization of multi-analytical systems will continue to grow with an increased focus on specificity, sensitivity, precision, and accuracy, resulting in increased efficiency in peanut contamination control.

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[D&PA 54]

Development of alternative “clean label” proposals to the use of preservatives and other additives in meat products

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Clean label products have been introduced in the market with the goal of ensuring consumer product safety having in sight the maintenance of environmental and public health (Delgado-Pando et al., 2021). This study aimed to assess if potential clean label solutions to nitrite addition are able to assure microbiological safety and confer a protective role against Clostridium spp. in novel ham formulations. The impact of these novel formulations on the human gut microbiome for potential consumers was also evaluated. Challenge testing was performed by artificial contamination of four different ham formulations with Clostridium sporogenes spores to assess their germination throughout 28 days at 4 °C and 10 °C, refrigeration, and abuse temperatures, respectively. The impact of these formulations on the human gut microbiome was assessed through HPLC quantification of short-chained fatty acids. No significant differences ($p<0.05$) in spore germination between ham formulations and the control were found for both temperatures. A 2 log and 2.5 log cycle differences were observed between day 0 and day 28 at 4 °C and 10 °C, respectively. Also, no significant differences ($p<0.05$) in the concentration of short-chained fatty acids were observed throughout 48 h of colonic fermentation in relation to the control ham, suggesting the absence of a negative impact of these novel products on the human gut microbiome.

These results demonstrate the potential of using natural sources of nitrates combined with nitrated-reducing starters as a clean label alternative to the use of nitrite in cooked ham, showing the promising application of this technology in day-to-day foods while having food safety in mind.

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[D&PA 55]

Plant-parasitic nematodes - a threat to food security

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Nematodes are a group of animals that can be found in soil, fresh water and marine environments as free-living nematodes, feeding on bacteria, fungi, protozoans and other nematodes, but there are species that parasitize animals, including humans, and plants. Plant-parasitic nematodes (PPN) can damage economically important crops, representing a significant threat to global food production systems and security, with worldwide losses of ≈173 billion US dollars/year [1]. The most notorious groups of PPN belong to the genera *Meloidogyne* (root knot nematodes), *Heterodera*/*Globodera* (cyst nematodes) and *Pratylenchus* (root lesion nematodes) [2]. According to the European and Mediterranean Plant Protection Organization (EPPO), some species (*G. pallida*, *G. rostochiensis*, *H. glycines*, *M. chitwoodi*, *M. enterolobii*, *M. fallax* and *M. mali*) are classified as quarantine pests, whereas *M. ethiopica*, *M. graminicola,* and *M. luci* are included in the EPPO Alert List [3,4]. These PPN have been detected in the EPPO region and present a phytosanitary risk. They occur frequently in agricultural systems and once established in the soil, their eradication is very difficult. The most effective strategies for PPN rely on the use of synthetic nematicides, however these chemicals present several environmental problems, their use has been heavily regulated and several products have already been banned from the market [5]. Detection, identification and knowledge on the distribution of PPN are essential not only to prevent their spread, but also to define sustainable management control strategies. The research work being carried out at the University of Coimbra (CFE and CIEPQPF) contributes to: 1) the characterization and identification of PPN, and 2) the development of sustainable strategies for its control, based on more environmentally-friendly use of natural-origin nematicides [6]. The knowledge generated is essential to prevent PPN spread and, by limiting the application of nematicides, to increase the efficiency of agricultural production system.

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[D&PA 56]

A rapid analytical method for determination of aminoglycoside residues by liquid chromatography and tandem mass spectrometry in muscle and milk

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The aminoglycosides (AGs) are a class of broad-spectrum antibiotics that are highly effective against Gram-positive and Gram-negative bacteria. They are widely used to treat bacterial infections such as bacterial enteritis and mastitis in veterinary medicine, but also as growth promotion. The European Union has issued strict Maximum Residue Levels (MRLs) for aminoglycosides in several animal origin products including milk and muscle [1].

This class of antibiotics presents significant challenges for residue analysis due to their high polarity with a tendency to undergo strong bindings to matrix proteins and also their poor chromatographic retention [2].

The proposed method has several advantages once it covers all relevant AGs at the required sensitivity and significant time and material savings have been achieved.

In this study AGs are extracted from muscle or milk with acetonitrile and aqueous trichloroacetic acid solution (5%) to precipitate protein and inhibit protein binding of the analytes.
Analysis was performed using a C18 column coupled to electrospray MS/MS, operated in positive mode, with detection by UHPLC-MS/MS. For the UHPLC analysis heptafluorobutyric acid (HFBA) was used as the ion-pairing reagent, compatible with mass spectrometry [3].

This methodology has been validated in accordance with the requirements for confirmatory criteria, described in the Commission Implementing Regulation (EU) 2021/808 [4].

The developed method will be applied in routine analysis of muscle and milk samples in order to control the abusive use of the referred class of antibiotics.

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[D&PA 57]

Impact of incorporation of insect-based flour on flatbread crackers on consumers' sensory perception and liking

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The topic of food unavailability has been discussed worldwide, with insects emerging as a promising alternative source [1]. However, despite the awareness of the benefits, several consumers are still not interested in adopting insects in their daily diet, based on concerns related to the safety of their consumption, sociocultural norms, and associations of insects with other sources of repulsion [2]. Preparing insects using techniques related to gourmet gastronomy, and incorporating them into familiar dishes and flavours where the animal origin is not visible, are some strategic methods that can be used to attract different consumers [3;4].

Following that knowledge, this study aimed to assess consumers liking of innovative food crackers, incorporating cricket (*Acheta domesticus*) flour. The sensory profiles of the different crackers were determined using a Check-All-That-Apply (CATA) approach. A panel of 50 consumers evaluated seven different formulations of crackers, differing on the type of herb used (Plain, Chives, Oregano, Basil, Rosemary, Fennel seeds and Sage), rating their overall linking using a 9-point hedonic scale. Following, consumers were asked to describe the samples using a CATA ballot with 33 sensory terms previously developed through bibliographic research.

Consumers showed that the samples presented a different sensory profile. Consumers generally liked the samples, with higher overall liking scores for the cricket flour crackers with Chives. This sample is characterized by its peppery, spices, toasted, salty and cereals flavours, with a crispy and crunchy texture. Within the sensory mapping, no significant differences were found between the Chives-based sample and the Plain sample.

The incorporation of cricket flour on crackers had a positive effect on consumers liking, revealing different profiles for the different formulations. The study provided valuable information on product development using insect-based proteins.

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[D&PA 58]

Is it necessary to use delivery vehicles for incorporating resveratrolinto bread?

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Trans-resveratrol (RSV) is a bioactive compound difficult to apply due to its poor water solubility and easy conversion into cis isomer. Delivery systems could overcome RSV constraints. Cyclodextrins (CDs) help to increase compounds' solubility by forming inclusion complexes [1] and zein nanoparticlesto improve bioavailability [2]. This work was intended to estimate the impact of using RSV complexed with γCD (γCD·RSV) and RSV-loaded zein nanoparticles (Npz-RSV) in functional wheat bread, on RSV stability, antioxidant activity, bioaccessibility and bioavailability.

The bread was prepared with wheat flour, water, yeast, salt and supplemented with 0.5% of RSV in freeform (control) and γCD·RSV and Npz-RSV. The γCD·RSV result of mixing an equimolar amount of an aqueous solution of γCD with an ethanolic solution of RSV, freeze-drying and characterization by spectroscopic analysis [3]. Npz-RSV were made by nanoprecipitation and properly characterized in load, size and zeta potential [4]. Bread were extracted with 70% ethanol which supernatant was injected for RSV quantification by ultra-high-pressure liquid chromatography at 306 nm and used on antioxidantactivity analysis regarding $ABTS^{\bullet+}$, oxygen radical absorbance capacity (ORAC) and nitric oxide (NO).RSV bioaccessibility was determined by in vitro digestion and bioavailability through cell modelpermeability assays.

γCD·RSV was composed by γCD.RSV.14H_2O. Npz-RSV had 187 nm, 23.2 ± 1.0 mV and loaded 13.9% of RSV. Bread extracts recovered 89.0%, 70.3% and 62.8% of RSV from RSV-, γCD·RSV- and Npz- RSV-bread, respectively, with some RSV retention due to the interaction of CDs and Npz with the breadmatrix. RSV granted antioxidant activity to bread, although some pro-oxidant activity of bread components and the quenching effect of RSV-delivery vehicles on chemical groups of RSV cannot be neglected [4]. γCD·RSV promoted RSV bioaccessibility (40%), but its bioavailability was not improved [5]. There is no need to use other delivery vehicles for RSV incorporation into the diet.

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[D&PA 59]

Bioprospecting *Laminaria digitata*-rich extracts as potential functional food ingredients

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Brown seaweeds are recognized for their abundance in phlorotannins, fucoxanthin and the polysaccharides fucoidans, laminarans, and alginates. Such compounds have been claimed to exert promising bioactivities which makes them attractive for the development of added-value products [1]. Nevertheless, the strong "fishy" odour and taste inherent to seaweeds limits its application e.g., as food ingredient, so using their extracts may represent a strategy to take advantage of their great value [2]. The present work aimed to obtain economic-affordable extracts rich in specific target compounds from brown seaweed Laminaria digitata to be potentially applied as bioactive ingredients.

A sequential solid-liquid extraction was developed consisting firstly of L. digitata powder' extraction with ethanol to recover phenolics and pigments. The resulting residues were extracted with hot water to recover polysaccharides, followed by precipitation with $CaCl_2$ 2% (w/v) to recover alginates. The phenolics and fucoxanthin were quantified by DMBA assay and UHPLC-DAD-ESI-MS, respectively, and polysaccharides were characterized in neutral sugars by GC-FID and uronic acids [3,4].

The ethanol extract contained 3% total phlorotannins and trace amounts of fucoxanthin. Besides, hot water fractions contained 52% total sugars, with major compounds being sulfates (41 mol%) and fucose (30 mol%), both associated to the presence of fucoidans, but also glucose (5 mol%) associated with laminarans. Also, calcium-alginates accounted for 6% algae dw showing M/G ratio of 1.5 as determined by HPAEC-PAD. At the end, algae extraction residues were still rich in alginates. To recover them, this residue was submitted to an additional extraction using Na_2CO_3. This process yielded 28% algae dw of sodium-alginates with slightly lower M/G ratio (1.4), compared to calcium-alginates. Overall, this work allowed the development of a sustainable extraction strategy to produce brown seaweed extracts rich in specific target compounds with bioactive potential that can be potentially used in the formulation of new functional products.

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[D&PA 60]

How to replace the white colorant titanium dioxide (E171) in foods?

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Titanium dioxide (TiO_2) is a ubiquitous white and opacifying agent, widely used in several areas, mainly in pharmaceutical, cosmetic, and food industries [1]. In Europe, TiO_2 is used as a food colour additive (E171), with the technological function of making food more visually appealing, providing a white colour to colourless foods, namely in sauces, icings, chewing gums, and candies. However, in 2022 European Food Safety Authority (EFSA) determined that TiO_2 is no longer authorized as a food additive, due to its potentially harmful effects to human health [2]. Consequently, European food industries are seeking for alternatives to TiO_2. Thus, the aim of this study was to develop alternatives to the white TiO_2 powder to be used in food stuffs. The strategy used was the consolidation of starch with an inorganic material, which consisted in swelling of the starch granules, and, consequently, decreasing the distance between inorganic particles, bringing them into contact to form a solid network [3]. Thus, rice starch was consolidated with different inorganic additives approved by EFSA, namely calcium carbonate (E170), calcium phosphate (E341), silicon dioxide (E551), and calcium silicate (E552), by gelatinization at 90 ºC during 30 min. The resulting white powders were then milled and sieved. As a showcase, the different white powders were applied in the recipe of commercial candies ("Flocos de Neve", Vieira de Castro – Produtos Alimentares, S.A.). The measurement of colour by CIELAB system (L*a*b* coordinates) revealed that candies produced with the addition of rice starch consolidated with silicon dioxide are more promised (L*=69), following by the mixture of rice starch with calcium silicate (L*=61), calcium phosphate (L*=53), and calcium carbonate (L*=48). In conclusion, the consolidation of rice starch with inorganic compounds revealed to be a good strategy as white food colour additives to replace TiO_2.

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[D&PA 61]

Application of an oral cell-based assay to study the organolepticproperties of *Clitoria ternatea* L. (Leguminosae) tea

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Astringency is a significant component of the sensory experience induced by a range of products. For beverages such as red wine, and tea, this sensation is desirable in balanced levels. However, in some foods such as fruits or vegetables, the same does not apply, making it a reason for rejection by consumers [3]. Astringency is described as dryness, and puckering sensation experienced in the mouth mainly induced by food polyphenols [1].

Polyphenols are compounds commonly found in various plant-derived foods known for their health benefits [6]. Anthocyanins, a complex group of flavonoids in the polyphenol family, are well-knownfor their color spectrum, which is strongly pH dependent [5]. In addition, anthocyanins have also recentlybeen shown to play a role in astringency perception.

Clitoria ternatea L. (Leguminosae), known as butterfly pea, has a characteristic bright blue color, which changes to light pink in weakly acidic solution and, in neutral and basic aqueous solution, remainsblue [4]. It is native to Southeast Asia and accumulates ternatins, a group of (poly)acylated anthocyanins[7]. The butterfly pea tea has been traditionally consumed for its medicinal properties [7]. It is described as having an herbal flavor that is likened to black tea and coffee [2] and able to induce astringency. In this regard, it is usually served alongside lemon and honey, giving it a milder flavor [2].

To decipher the mechanism beyond butterfly pea tea astringency, this work aimed to study the interaction of the compounds in butterfly pea to various oral constituents. The study was carried under a cell-based model containing the main oral constituents involved on the astringency perception: humansaliva, mucosa pellicle, and an oral cell line (HSC-3). Overall, the results revealed a more significant interaction of the compounds with the oral cells and with mucosa pellicle + mucous membrane film.

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[D&PA 62]

Does Laminaria digitata has potential to alleviate drought stress in tomato plants?

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The agrifood sector is facing the acceleration of climate change episodes, such as drought, with consequent reduction of crop yields. These challenges urge developing new sustainable strategies enabling crops to combine high productivity and tolerance to abiotic stressors. This work focused on the benefits of the brown seaweed *Laminaria digitata* aqueous extract (*LE*) against drought effects. *Solanum lycopersicum* (tomato) plants were foliar sprayed with solutions of 0, 0.1, and 1 g/L of LE for 3 times and, afterwards, were divided in two groups: water stressed (WS) and well-watered (WW). The WS group wasn't watered for a week while the WW group continued being irrigated. After this period, the leaves were analyzed for their morphology and physiology.
The LE contained 27.7 % ash, 4.8% protein, and 38.5 % of total sugars. The foliar application of the extract did not change the tomato plant growth, leaf water content, neither proline or H_2O_2 contents. Nevertheless, *LE* stimulated gas exchange in WW, with the increment of the net CO_2 assimilation rate, stomatal conductance (g_s), intracellular CO_2 concentration, and transpiration rate (*E*). Overall, there was a decrease of g_s and *E* in both concentrations and P_N in the highest dose, in WS. Despite that, the water use efficiency showed a trend to increase in this group, contrarily to the WW one. Concerning the fluorescence of chlorophyll *a*, a trend to increase the maximum efficiency of PSII was detected in WW, despite a decrease of the effective efficiency of PSII (Φ_{PSII}), accompanied by the increase of the photochemical quenching (qP) and the decrease of the non-photochemical quenching at the lowest dose. At the same dose and in WS, the treatment enhanced the Φ_{PSII}, together with the increase of qP. These results pinpoint *LE* potential to increase drought tolerance and as plant biostimulant.

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[D&PA 63]

Evaluation of 125 pesticides residues by HPLC tandem mass spectrometry in Portuguese brassica vegetables

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In recent years, Brassica vegetables have acquired the reputation of ´superfoods' due to their content of glucosinolates, bioactive sulfur-rich compounds associated with their health benefits [1].
Pesticides are most commonly employed to protect plants from pathogenic organisms and pests. Agricultural activities are frequently blamed for pesticide contamination in the environment.
In the present study, a method was developed and validated to determine pesticide residues in brassicas. Pesticide residues were determined in brassicas using the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique, followed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). A total of 125 pesticides were validated according to the guidance document SANTE/11312/2021 [2]. The method was linear in the range 5-50, 5-60, 5-70, 5-80 and 5-100 µg/kg, depending on the evaluated pesticides. The methodology was precise (3.9-13.5%) and accurate (recovery range between 75-104%).
Twenty-three brassicas from different varieties were acquired in local supermarkets of the Porto region (Portugal) and obtained from in-house/local producers. The maximum residue limit has been exceeded for some pesticides in kale samples from in-house producers (not available for acquisition), such as ethoprophos (0.02 mg/Kg), which maximum permitted level is 0.01 mg/Kg [3]. These results underline the importance of information on good agricultural practices in pesticide application. In the future, the effect of food processing on the pesticide content of brassicas will be evaluated.

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[D&PA 64]

Validation of an HPLC-MS/MS method to determine pesticides residues in berries and its application to strawberry samples

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Pesticides are widely employed to protect crops; however, their overuse/misuse, particularly in developing countries, volatility, and long-distance shipping, all contribute to broad environmental damage [1]. To limit pesticide use and reduce undesirable side effects, the European Union (EU) agreed to establish maximum residue levels of pesticides in food and feed of plant and animal origin [2]. Strawberry is a commercially important crop grown and consumed worldwide, and the number of countries producing it is increasing, as seen by an increase in global production from 7.6 million kg in 2014 to 8.9 million kg in 2019 [3].

This study aims to develop and validate a method for determining pesticide residues in berries. QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) was chosen as the pesticide extraction method. The extracts were further analyzed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). For this study, 92 pesticides were validated, according to the guidance document SANTE/11312/2021 [4]. The methodology was found to be accurate (3.4-10%) and precise (recovery range 75-94%), with an effective application suitable for assessing a broad spectrum of pesticides in berries.
Eleven samples were obtained from local supermarkets in the Porto region (Portugal) and in-house/local producers. Regarding the analysed samples, only one sample exceeded the maximum residue limit for pesticide residues. In this sample (obtained from an in-house producer), cyprodinil was found at a level of 0.72 mg/Kg, exceeding the maximum residue limit of 0.10 mg/Kg.
In this study, the samples under analysis did not undergo any water-based processing, so it would be expected that washing application would impact the residue levels detected. We are planning a study to evaluate the effect of different processing/cooking methods on the pesticide residues content of strawberries.

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[D&PA 65]

The effects of fermentation time and pasteurization methods on the production of Opuntia ficus-indica fermented beverages

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Opuntia ficus-indica fruits possess high levels of micronutrients and phytochemical compounds, such as betalains and phenolic compounds, therefore presenting a high nutritional value and potential health benefits, namely in the prevention of several diseases [1, 2]. The pulp is characterized by a high water content, reducing sugars (10%- 15%), 0.3% ash, and less than 1% protein [3]. The high soluble solids content and the pH of approximately 6 make prickly pear pulps a very attractive medium for the growth of microorganisms [4], requiring treatment to control their growth. Thus, this work resorted to alcoholic fermentation of centrifuged prickly pear juice and posterior pasteurization to ensure its microbial safety. The juices were fermented for 18 h (F18) and 42 h (F42) and two pasteurization processes, namely temperature (TP, 71.1 ºC for 30 s) and high-pressure (HP, 500 MPa for 10 min) were applied to the fermented beverages. In addition, physicochemical parameters, namely pH, titratable acidity (TA), ºBrix, browning and turbidity, were analysed. Globally, F42 was shown to be preferential, resulting in a beverage with lower pH and high TA - characteristics that prevent microbial growth; and a lower browning and turbidity - that are considered consumer acceptance factors. Furthermore, enzymes such as polyphenol oxidase (PPO), pectin methylesterase (PME), and peroxidase (POD), were measured after HP and TP processing. The enzyme PPO showed a reduction in its activity of approximately 48% and 65% when compared to the non-pasteurized juice and the processed samples, respectively. Complete inactivation of the PME enzyme was achieved in the samples subjected to thermal and high-
pressure pasteurization.
In general, a F42 and high-pressure processing proved to be effective in maintaining the stability and overall quality of the desired end product, thus suggesting a possible market acceptance of a new fermented beverage with similar characteristics to a traditional cider.

Table 1. The effects of fermentation time and pasteurization methods on the physicochemical parameters of *Opuntia ficus-indica* cv 'Rossa' fermented beverages

Fermentation time (h)	Sample	pH	Titratable acidity ($g_{citric\ acid}$/L)	Total soluble solids (°Brix)	Browning	Turbidity	Reducing sugars (mg_{sugar}/mL)	Alcohol content (% (v/v))
NA	Juice	5.84 ± 0.14^{a}	0.049 ± 0.002^{a}	13.10 ± 0.21^{a}	1.139 ± 0.082^{a}	0.432 ± 0.004^{a}	158.87 ± 18.07^{a}	*
18	NP	4.15 ± 0.04^{b}	0.393 ± 0.010^{b}	8.60 ± 0.00^{b}	0.816 ± 0.025^{b}	1.724 ± 0.034^{b}	62.34 ± 5.13^{b}	2.494 ± 0.000^{a}
	HP	4.11 ± 0.03^{b}	0.178 ± 0.004^{c}	8.60 ± 0.00^{b}	0.837 ± 0.011^{b}	0.970 ± 0.001^{c}	49.56 ± 2.55^{b}	2.494 ± 0.000a
	TP	4.31 ± 0.02^{c}	0.222 ± 0.008^{d}	7.67 ± 0.12^{c}	0.768 ± 0.009^{b}	1.969 ± 0.069^{d}	39.88 ± 1.62^{b}	2.975 ± 0.076^{b}
42	NP	3.91 ± 0.03^{d}	0.494 ± 0.005^{e}	3.93 ± 0.12^{d}	0.936 ± 0.004^{c}	0.518 ± 0.025^{ea}	2.123 ± 0.536^{c}	4.900 ± 0.076^{c}
	HP	3.84 ± 0.02^{d}	0.283 ± 0.007^{f}	4.13 ± 0.06^{d}	0.794 ± 0.005^{db}	0.076 ± 0.006^{f}	2.139 ± 0.676^{c}	4.856 ± 0.000^{c}
	TP	4.10 ± 0.03^{b}	0.328 ± 0.020^{g}	4.53 ± 0.12^{e}	0.704 ± 0.001^{eb}	0.160 ± 0.011^{f}	3.120 ± 1.265^{c}	4.637 ± 0.076^{d}

*The values represent the mean (N=3) ± SD

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[D&PA 66]

Improvement of GABA content by rice solid-state fermentation

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Portugal, the highest rice consumer in Europe, has twice the average European rice consumption. γ-aminobutyric acid (GABA) is recognised for its potential to lower blood pressure [1]. This non-protein amino acid is present in many food matrices, including rice, and can be improved by processes such as germination or fermentation, as during those processes glutamic acid is decarboxylated to GABA in the presence of glutamic acid decarboxylase (GAD). Rice solid-state fermentation with *Aspergillus Oryzae*, known as Koji [2], is very common in Japan [2] and it's used as a starter for many traditional foods, including soy sauce, miso and sake [3].

Steam cooked rice (milled, brown and germinated) from the Ariete variety was fermented under standardized conditions of moisture and inoculum, considering fermentation time and temperature as variables for GABA optimisation, by using a response surface methodology. GABA content in each surface point was analysed by HPLC-RP after extraction with ethanol followed by HN derivatisation and quantified by using an external calibration curve. Starch, which is the main compound of rice, was also measured in the same fermented samples, by using Near Infrared Spectroscopy (NIRs), to understand the changes of the matrices during the fermentation process.

The results showed the germinated rice had the greatest GABA content (289.9 g/100 g of Koji), at 32 ºC/60 h fermentation. Brown and milled rice presented relatively lower values of GABA (247.6 and 266.3 g/100 g respectively) both at 37 ºC and 40 h to 44 h of incubation. The response surface plots show the germinated sample greatly modified the food matrix, which is also visible in the starch content plot. Starch presented a lower content in the surface plot areas with greater GABA contents, in all matrices, showing fermentation partially hydrolyses starch during the process through the action of α-amylase.

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[D&PA 67]

Survival of bacteriocinogenic cultures of lactic acid bacteria in radish sprouts

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Soil is a potential source of plant contamination with foodborne pathogens, which can lead to foodborne disease, especially through the consumption of ready-to-eat products. In order to reduce potential contamination in these foodstuffs, the use of lactic acid bacteria (LAB) has emerged as a potential strategy for the biocontrol of foodborne pathogens.
The main objective of this work was to evaluate the survival of bacteriocin-producing LAB throughout the germination of radish sprouts (Raphanus sativus L.).
Pediococcus acidilactici HA-6111-2 was inoculated into radish germinates at 10^5 and 10^8 colony-formingunits (CFU)/g. LAB counts were performed on MRS immediately after inoculation (day 0) and after 6 days at room temperature, corresponding to the complete radish germination period. Uninoculated germinates were used as a control.
After complete germination (day 6), an increase of approximately 3 log cycles was observed for P. acidilactici inoculated at 10^5 CFU/g. At the end of the germination stage (day 6), P. acidilactici inoculatedat a higher level (10^8 CFU/g) maintained the initial cell numbers. No LAB were found in the control radish germinates. Moreover, P. acidilactici had an apparent slight impact on the cultivation of the germinates,promoting their growth.
Although preliminary, these results point to the survival of bacteriocin-producing LAB in radish germinates. Further tests regarding the potential of these LAB as biocontrol agents against important foodborne pathogens, as well as their ability to fulfil their role as probiotics and growth promoters, shouldbe assessed to develop safe and functional sprouted products.

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[D&PA 68]

Impact of nitrite on the microbiological quality of a Portuguese Salpicão

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The development of healthier traditional product formulations is required by new trends in the food industry. Meat curing is widespread throughout the world and is an integral part of traditional gastronomy. The use of nitrite salts is paramount in suppressing lipid oxidation and reduce growth of pathogenic bacteria. This activity is especially significant when inhibition of Clostridium botulinum is necessary, inhibiting vegetative cell proliferation and germination of spores. While this is widely recognised, the impact of nitrite on the total microbiota of fermented meats is not yet fully understood.

The ingestion of excessive nitrite has been correlated with an increased risk of cardiovascular pathologies, diabetes, blood diseases, such as infant methemoglobinemia, and cancers. As a result, a popular adverse reaction to the use of nitrite salts has been trending.

With the advent of NGS (Next-Generation Sequencing) techniques, detecting changes in microbial communities can be performed with unparallel specificity. Therefore, the evolution of microbiological diversity as a result of nitrite elimination during the different processing phases of Salpicão was analysed in this work (raw meat T0; before stuffing T1; middle of curing process T2; finished product T3; middle of shelf-life T4; and end of shelf-life T5).

Variations in the microbiota composition were detected, with certain genera, such as Pseudomonas, Lactobacillus, and Weissella showing resistance to nitrite, evidenced by their growth throughout the process, while Brochothrix and Salmonella appear to be more susceptible to its activity. Regarding the main target of nitrite, Clostridium, this bacterium was found in lower abundance in samples with nitrite (0.02%) than in nitrite-free samples (0.23%). Therefore, nitrite seems to be effective preventing Clostridium spp. growth, while also reducing microbial levels of other undesirable bacteria.

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[D&PA 69]

Sensory dynamic characterization of clean label mayonnaise using projective mapping

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Projective mapping is a fast sensory profiling technique where participants compare samples, considering similarity criteria [1]. Using this approach, the present study aims to analyse consumer perception and characterization of a set of twelve mayonnaise formulations varying on the degree of innovation and clean label ingredients. The sensory analysis tests were performed by 80 consumers, according to the following stages: (i) the projective mapping of the full sample set was carried out; (ii) ultra-flash profiling was used as a complement, which allowed the characterization of the products facing different sensory attributes, (iii) overall liking of the products was also collected.

From the overall liking results, three clusters of consumers were identified, which tend to perceive the samples in different ways. For the first group, product liking is mostly associated with samples with a creamy and consistent texture, smooth and optimal intensity taste. The second group tends to value products with a consistent texture and a whitish appearance. Finally, the third cluster assumes an intermediate position between the first two groups, valuing a mayonnaise with a creamy texture, whitish appearance, and mild flavour.

The information collected allowed to better understand that liking of mayonnaise samples tends to be related to attributes such as: smooth and creamy texture, light colour, soft and sour, typical, balanced, vinegary and mild taste. On the other hand, the sample least liked by consumers is predominantly characterized as a product with a greasy and sandy texture and an atypical, neutral, and slightly acidic taste.

This research brought important aspects about this food product, allowing to characterize the sensory profile of this mayonnaises. It was also interesting to note that differences in overall liking were connected to distinctive sensory attributes.

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[D&PA 70]

Development of Clean Label Bakery Products with natural preservatives

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Bakery products are, in general, highly appreciated and demanded worldwide, both for their organoleptic characteristics as well as for the versatility they provide. However, the nutritional imbalance and high energy density of some formulations have increasingly promoted the connotation of many bakery products with unbalanced dietary patterns and their relationship with metabolic syndrome and chronic non-communicable diseases (NCDs). Besides, the need to extend shelf-life and improve some sensory properties have forced the increasing use of synthetic additives.

In this sense, the objective of this work was to reduce the number of synthetic additives in these products, creating a new line of "*Clean Label*" products, and replacing preservatives, aromas, and synthetic dyes with natural alternatives, wherever possible. Thus, the antimicrobial activities (minimum inhibitory concentrations; MIC's) of five plant/fruit extracts (namely, savory, marigold, orange, turmeric, and acerola) were analysed and new "*brioche*" dough formulations were developed replacing the synthetic preservatives with the best natural antimicrobials.

Results showed that both turmeric and acerola at 0.75 g / L had antimicrobial activity against *Bacillus cereus* and *Bacillus subtilis* but not against *Saccharomyces cerevisiae*. The new formulations showed no negative impact on dough properties and sensory quality. Furthermore, the inclusion of alternative natural ingredients conferred an extended shelf-life to the products, in comparison with the ones manufactured with the standard/control formulation, responding to the "Clean lable" concept.

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[D&PA 71]

Production of a more Diabetes-friendly granola by incorporation ofa phlorotannins-rich extract

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Diabetes mellitus is a chronic disease that leads to a degenerative and debilitating condition in the patient's quality of life. Prevention is the best way to fight this disease through healthy eating habits. Macroalgae has been reported as a rich source of bioactive compounds, namely phlorotannins with beneficial effects in the prevention of diabetes and a great potential for the application in new functionalfoods [1]. The aim of this work is the development of a functional granola, enriched with a phlorotannins-rich extract from the brown macroalgae Laminaria digitata. The UHPLC-ESI-MS analysis at 280 nm revealed a chromatographic profile characteristic of phlorotannins, showing an unresolved hump typical of unseparated higher molecular weight compounds preceded of small peaks corresponding to oligomeric phlorotannins (fig.1).

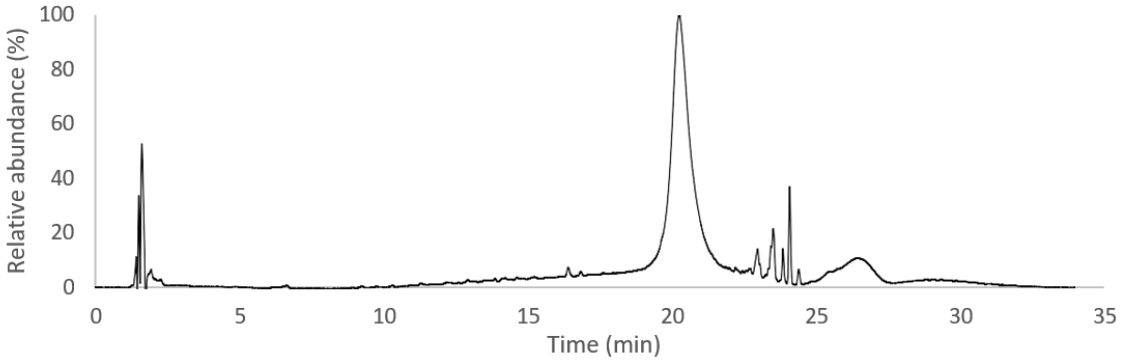

Figure 1. Chromatographic profile of *Laminaria digitata* ethanol extract at 280 nm.

The antioxidant activity measured by the $NO^{\bullet}$ and $O_2^{\bullet -}$ scavenging methods and the inhibitory effect on α-amylase and α-glucosidase was also evaluated. Interestingly, the inhibition activity against α-amylase was similar to acarbose and against α-glucosidase was approximately 200-fold higher than acarbose and 3-fold higher than other phlorotannin-rich extracts from *Fucus vesiculosus* [2]. Adding this this phlorotannins-rich extract to granola (2%) got a good acceptance in the sensory analysis and contributed to the inhibition of both enzymes, especially α-glucosidase. This work shows that the phlorotannin-richextracts of *Laminaria digitata* have great potential to be used as an ingredient for the development of functional foods that can help prevent diabetes.

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[D&PA 72]

The effect of pine bark extract on *Chouriça*'s lipid oxidation

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Bark from *Pinus pinaster* Aiton subsp. *atlantica* is an abundant waste from the timber industry. There is an increasing interest in its use since it is an important source of natural polyphenolic compounds with well-known abilities to scavenge free radicals, i.e., antioxidant power. In this study, the antioxidant effect of pine bark extract on lipid oxidation in "Chouriça", a sausage-like Portuguese traditional meat product, was analysed.
Three batches of "Chouriça" were produced in collaboration with a meat processing industry, MinhoFumeiro. For the ChM batch, pine bark liquid (PBE) extract was added while mixing meat with wine and spices before being subjected to thermal processing (smoking). PBE was added to batch ChP after thermal processing by brushing the surface. Control samples (ChC) were produced as usual with no extract addition. Samples were packed in a modified atmosphere and stored at 4 ºC before analysis. The lipid oxidation was measured (TBARS, acidity, peroxide value) in samples from the three batches 10 days after the Chouriça's processing was concluded.
Relevant statistical differences ($p<0.05$) were found between control samples (ChC) and the ones with PBE addition. ChC had the highest values for TBARS, acidity and peroxides (7.30±0.07 mg/g, 3.89±0.34% and 43.75±6.69 meq/kg, respectively). ChM had lower TBARS and peroxide values than ChP ($p<0.05$, 4.21±0.07 and 5.36±0.13 mg/g for TBARS and 18.92±28.81 and 28.81±0.54 meq/kg, respectively). No differences were found in acidity between ChP and ChM.
In this preliminary study the addition of PBE delayed lipid oxidation, especially if applied before thermal treatment. Furthermore, PBE appears to protect "Chouriça" against early fat oxidation, which can be useful to extend the shelf life of these meat products. Also, applying natural bioactive compounds to meat products is an opportunity to develop sustainable food products adapted to current consumption trends, valorising underexploited forestry by-products.

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[D&PA 73]

The application of Pine bark extracts to improve the antioxidant activity of Atlantic Horse Mackerel dried fillets

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Modern consumers are interested in natural convenience foods with high nutritional value, having additional biological and health benefits. Fish is one of the most nutritious foods yet a highly perishable product with a very short shelf- life. Bark from *Pinus pinaster* Aiton subsp. *atlantica* is a source of phenolic extracts, with known antioxidant and antimicrobial activities, having great potential for application in foods. This study determines the effect of adding pine bark extracts to brine Atlantic horse mackerel (*Trachurus trachurus*) fillets, aiming to develop an innovative functionalised product enriched with natural bioactive compounds.
Fish skin-on fillets were brined with three different formulas: 5% salt (A), 5% salt+20% liquid smoke (B), and 5% salt+20% liquid smoke+200 µg/mL pine bark extract (C), at 10 ºC for 30 minutes. Fillets were dried (70 °C, 3 h) in a mechanical chamber. Chloride content (in the form of NaCl), moisture content, total phenolics content, anti-radical capacity DPPH, ABTS and ORAC parameters were determined in triplicate. Statistical differences were measured using one-way-ANOVA followed by a *posthoc* Tukey test.
Sample A presented the higher chloride content (2.24±0.02 g/100 g), and no differences ($p=0.14$) were found in samples B and C (1.34±0.01 g/100 g and 1.39±0.04 g/100 g, respectively). Relevant differences ($p<0.05$) in moisture content were found in sample C (65.91±0.22%), and samples A and B showed no differences. Higher contents ($p<0.05$) of phenolic compounds were found in sample C (0.74±0.00 mgGallic acid/g fish, versus 0.52±0.01 from sample A and 0.56±0.00 from sample B). The antioxidant effect was also higher ($p<0.05$) in samples with the addition of pine bark extract: 167.18±0.33 mgTroloxEq/g in ORAC, 27.61±0.16 mgTroloxEq/g in ATBS, and 12.20±0.05 mgTroloxEq/g in DPPH determinations.
Pine bark extracts incorporated into horse mackerel fillets is an opportunity to develop enriched sustainable food products, adapted to current consumption trends, valorising underexploited aquatic and forestry by-products.

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[D&PA 74]

Effect of Brazilian *Spirulina* on rheology and texture properties of emulsions

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The use of innovative and sustainable ingredients, such as *Spirulina* biomass (SB), has been stated as a fact of food products development, mainly to guarantee foods with better technological, nutritional, and sensory quality, in addition to the positive impact on the environment, natural resources, and animals, to ensure sustainable food systems. *Spirulina* (Arthrospira platensis) is a filamentous cyanobacterium, popularly known as microalgae, with high nutritional value, as a source of proteins, and bioactive compounds, particularly natural pigments such as C-phycocyanin (C-PC). C-PC is a natural blue phycobiliprotein that, in addition to its function as a pigment, has valuable features with beneficial biologic effects, attracting commercial interest in the pharmaceutical, food, and cosmetic industries. However, there are several challenges concerning using natural pigments effectively in foods due to their instability related to different conditions regarding the digestion physiology and the difficulties in front of the processing and storage of foods using C-PC.

Therefore, this study aimed to develop emulsion by adding Spirulina and C-PC. Compared to the control, the impact of these incorporations on the emulsion rheological performance (pasting curves and viscoelastic behavior) was performed.

Batches of 100 g of emulsions were produced. Before the emulsion production, the vegetable proteins (chickpea) were dispersed in distilled water for 30 min under magnetic stirring, at room temperature, for hydration. Emulsions were stabilized with 3.0 % (w/w) of total protein. Once the protein was dispersed and hydrated, sunflower oil (65 % w/w) was added under agitation in an Ultra Turrax T- 25 (IKA, Germany) homogenizer, at 14 000 rpm, for 10 min, according to previously optimized conditions, for a similar system. The results have shown that Spirulina and C-PC addition affected the rheology properties of the emulsions. Further analyses are necessary to comprehend better Spirulina and C-PC emulsions sensorial acceptance and proximate composition.

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[D&PA 75]

Formulation of green ice cream using organic and biodynamic *Spirulina*: the study of color stability

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Society has shown itself to be more concerned with healthier habits, and accompanied by this, it is more demanding in the search for natural and healthy foods. In this context, the study of bioactive compounds and their health benefits has become increasingly relevant. *Spirulina* is a microalgae known in the literature for its rich nutritional composition and as a source of bioactive compounds. It has a high protein content, polyunsaturated fatty acids, tocopherol, chlorophyll, carotenoids, and C-phycocyanin (C-PC). The pigmentation and beneficial health properties of C-PC illustrate its high commercial value and notability as a substitute for artificial food colors. Therefore, in view of the characteristics of Spirulina and its great potential as a substitute for artificial colors in foods, the main objective of this study is to evaluate the color stability of a yogurt ice cream with *Spirulina* added as a source of color and bioactive compounds.

Spirulina biomass was donated by Fazenda Tamanduá® (Santa Terezinha/Paraíba – Brazil) and was stored in a freezer (-40 °C until its use). Analytical colorimetric analysis was performed using a Colorimeter (Minolta, model CM25D, Japan). The values of L*, a*, and b* were determined. The Hue angle (h) indicates the color angle (0°- red, 90°- yellow, 180°- green, 270°- blue, and 360°- black). L* indicates brightness (0 - 100), a* indicates red (positive values) or green (negative values), and b* shows yellow (positive values) or blue (negative values). The color difference (ΔE) was calculated from the initial (0) and final (t) values for the ice cream formulation with time.

The results showed color stability considering the evaluated time (1 month) and are a starting point for new products containing natural pigments with functional appeal. Further research should assess sensorial acceptance and antioxidant activity.

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[D&PA 76]

Development of an UHPLC-TOF-MS Methodology for Determination of Tropane Alkaloids in Buckwheat and Buckwheat Products

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Buckwheat is a pseudocereal, and recently became a trendy food due to its good nutritional profile and being gluten-free [1,2]. Tropane alkaloids (TAs) are natural compounds produced by plants that can accidentally occur in the food chain due to contamination with seeds, especially from *Datura* L. plants, since they have a similar size of buckwheat [3]. TAs, like atropine and scopolamine, are toxic for humans, when ingested at high doses, with adverse effects on the cardiovascular system [4].

This study developed an Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) method to determine atropine, scopolamine, anisodamine, and homatropine in buckwheat and buckwheat products, through a QuEChERS approach.

The analytical methodology was successfully validated, demonstrating good linearity, sensitivity (Limits of quantification, LOQ, between 0.625 and 1.25 µg/kg), accuracy (recovery between 74 to 113%), repeatability (RSD_r < 15%), and precision inter-day (RSD_R < 19%) in accordance with criteria established by Commission Recommendation EU No. 2015/976. This method allows the determination of TAs, since LOQ is lower than the maximum levels regulated by the EU.

The developed method was applied to monitor the presence of TAs in thirteen samples of buckwheat-based products, detecting atropine in one sample (2.13 µg/Kg) and homatropine in two samples (1.92 µg/Kg and 0.98 µg/Kg). The concentration of atropine was lower than maximum permitted levels at European Union. Scopolamine and anisodamine were not detected above the established limits of detection.

In conclusion, the optimized analytical method is reliable, accurate and its low detection limit allows to evaluate the compliance of buckwheat with EU maximum permitted levels of TAs. Buckwheat-based products available in Portuguese markets are safe regarding these plant toxins, although the evaluation of more buckwheat samples should be carried out. Furthermore, this method could be applied in other pseudocereals like quinoa or amaranth, which might be contaminated with TAs and whose consumption greatly increased in recent years.

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[D&PA 77]

A Novel Approach for the Quantitative Analysis of Tropane Alkaloids in Herbal Infusions

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Herbal infusions are highly popular beverages consumed daily due to their health benefits and antioxidant properties [1]. However, the presence of plant toxins constitutes a recent health concern in herbal infusions [2]. Tropane alkaloids (TAs) are natural molecules found in plants that may unintentionally enter the food chain as a result of contamination from Datura L. plants [3].

This work optimized and validated a methodology based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction procedure followed by Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) for the determination of tropane alkaloids (atropine, scopolamine, anisodamine, and homatropine) in herbal infusions.

The method provided good analytical performance, with good recovery (82 to 105%), repeatability (RSDr < 11%), and precision inter-day (RSDR < 13%) in agreement with criteria established by Commission Recommendation EU No. 2015/976. The limit of quantification for atropine and for scopolamine was 5 µg/kg (for each molecule individually), which is lower than the maximum levels regulated by the EU (25 µg/kg for the sum of atropine and scopolamine).

The validated method was used to assess the presence of TAs in 17 dried herbs used for the preparation of infusions, purchased in Portuguese supermarkets. One of these samples was contaminated with atropine (38.11 µg/Kg), corresponding to a lemon balm sample (Melissa officinalis) exceeding the current EU regulations. Also, homatropine was found in stinging nettle (Urtica dioica) at a concentration of 10.98 µg/Kg, although this TA does not have an established maximum permitted level.

In summary, the results indicate the importance of monitoring TAs in dried herbs to avoid possible adverse health effects in consumers of herbal infusions. Also, the determination of TAs in the infusions prepared with these dried herbs should be evaluated to assess the influence of the different conditions of infusions' preparation (e.g. temperature and time) in the "migration" of TAs from the dried herb to the infusion.

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[D&PA 78]

Improving the sensory profile of microalgae using ultrasound extraction: using the bioactive-rich fraction in vegan o/w emulsions

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Microalgae are a remarkable source of bioactive compounds and nutritionally rich in proteins, minerals, vitamins, lipids and dietary fibres [1], but not yet a common food ingredient. The main bottlenecks for the industrial scale-up are related to the fish-like smell and taste associated with volatile compounds [2]. Their use as sustainable food ingredients may require clean pre-treatment processes that will improve the consumers acceptance. This work is part of Project DoMar, and ultrasound (US) extraction was evaluated as a valuable technique to improve the microalgal sensory profile while maintaining bioactive properties.

The US-assisted extraction was carried out using an UP200Ht ultrasonic processor (Hielscher) with a S26d26 sonotrode half-submerged in 100mL of microalgal solution, power of 190W. *Microchloropsis oceanica* and *Dunaliella salina* concentration (dried powder) was 1% and 5% (w/v) in 96% ethanol and 60% of ethanol/water. The acoustic energy supply was continuous, amplitude set at 100%, the extraction time was 5 min and intend to promote the full recovery of the two fractions (solid and liquid), in a circular economy approach. Volatile compounds composition and sensory results of both fractions, in comparison with raw microalgae biomass, showed a reduction of the undesirable smells from the microalgae, with different impacts on total phenolics and antioxidant capacity.

Within the project, it is intended to fully recover the two obtained fractions, in a circular economy concept. The solid protein-rich fraction will be incorporated into bread, and the bioactive-rich fraction will be tested as an emulsifier agent. In the present work, the bioactive-rich fractions obtained by processing *M. oceanica* and *D. salina* using US at selected conditions were incorporated into oil-in-water emulsions stabilized by fava (2% w/w) and lupin (2% w/w) protein isolates. The mechanical properties and consumer acceptance of the microalgae-enriched mayonnaises were investigated.

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[D&PA 79]

Using enzymes to improve microalgae sensory quality? Impact on cheese and bread

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The food system transition is one of the biggest challenges of our time. In the context of climate change and food shortage, we need to produce sufficient nutritious food to enable positive health impacts, using sustainable production methods. Microalgae are already considered a food for the future due to their richness in high-valued nutrients and their contribution for the transition to a carbon-neutral economy [1].

However, the microalgae addition in food formulations is a challenge, given its impact on the food structure that can lead to changes in the rheological behavior. In addition, the incorporation of microalgal biomass induces changes in the color and flavor, namely the fish-like smell associated with volatile compounds [2,3]. There are some microalgae with less pronounced colors and flavors, produced in heterotrophic conditions, which is restricted to some species, and their bioactivity is lower compared to autotrophic microalgae. Other technologies have already been applied, such as the extraction with ethanol, but also with a negative impact in the bioactive properties [4]. Therefore, it is essential to bet on clean technologies, which in the future can be used by microalgal biomass producing companies.

The YUM ALGAE project aims to improve the overall sensory quality of microalgal ingredients by using enzymes, which will target their usual bottlenecks: strong odor, taste and color. Two different food structures are studied: bread, a dough-based system subjected to severe thermal treatment; and cheese, a gelled structure prepared using pasteurized milk. Both foods are processed by fermentation, which can positively enhance their sensory profile and balance the algae contribution.

In the present work, the results of the technology, nutritive and sensory quality of the microalgae-added cheese and bread products will be presented. Microalgae produced within the project can be used as natural and innovative ingredients to nutritionally enrich the contemporary diet and encourage sustainable consumption.

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Dare 2 Change

Health and Wellbeing

Acheta domesticus as a source of bioactive peptides with anti-hypertensive, anti-diabetic, anti-pulmonary and cardiac fibrosis properties: an *in silico* approach

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Entomophagy is being proposed as a sustainable source of proteins in alternative to traditional animal meat (e.g., beef, poultry, pork) [1]. In addition to their nutritional value, insects are also a source of bioactive peptides with anti-hypertensive, anti-diabetic, anti-obesity and anti-oxidative properties [2]. Although it is estimated that more than 2000 insect species are consumed worldwide as food [3], to date the European Union (EU) has only authorised the commercialisation of three insect species that comply with the Regulation (EU) 2015/2283 on novel foods. *Acheta domesticus,* known as house cricket, is one of the EU regulated species. It produces a nutrient rich flour and is easy to farm [4], but to date, there are no studies devoted to the identification of its bioactive peptides.

In this study, an *in silico* approach was applied to identify and characterise bioactive peptides resulting from the simulated gastrointestinal digestion (GI) of six *A. domesticus* proteins. The anti-hypertensive and/or anti-diabetic bioactivities of peptides were assessed through a molecular docking protocol to evaluate their binding interactions with two catalytic domains (N- and C-) of the somatic angiotensin-I converting enzyme (sACE) and with dipeptidyl peptidase 4 (DPP-4), respectively.

The digested proteins originated 43 peptides that were ranked with a probability >50% of being bioactive. Five of them (AVQPCF, CAIAW, IIIGW, DATW and QIVW) showed high docking scores for both enzymes, suggesting their potential to inhibit the DPP-4 and both sACE domains, thus possessing multifunctional bioactive properties. Two peptides (PIVCF and DVW) showed the highest docking scores for the N-domain of sACE, indicating a potential action as domain selective inhibitors and, therefore, possessing anti-cardiac and anti-pulmonary fibrosis bioactivities. This is the first study identifying bioactive peptides originated from the simulated GI digestion of *A. domesticus* with multifunctional potential activities against hypertension, diabetes, cardiac and pulmonary fibrosis [5].

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Actinidia arguta leaves potential nutraceutical ingredient: an *in vitro* and *in vivo* screening

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Actinidia arguta leaves are a source of phenolic compounds with pro-healthy biological effects, such as antioxidant and anti-inflammatory activities [1]. This study aims to validate by in vitro and in vivo assays an A. arguta leaves extract as new nutraceutical ingredient.
A. arguta leaves were extracted with ultrasound-assisted extraction, according to Silva et al. [2]. A 3D intestinal permeation was performed using a Caco-2 and HT29-MTX co-culture, accoupled to LC/DAD- ESI-MS analysis. For the in vivo assays, Wistar rats (n = 6/group) were orally treated during 7 days with water (Group I), A. arguta leaves extract (50 and 75 mg/kg bw/d, respectively, Group II and III) or vitamin C (Group IV). The animals were sacrificed, the liver and kidneys were removed, and the blood collected. The antioxidant enzyme activities, namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were determined.
The LC/DAD-ESI-MS analysis revealed that coumaroyl quinic acid was the principal phenolic compound identified and quantified in all time points, achieving an intestinal permeability of 34.23% at the final time point (240 min). Rutin achieved the highest permeability at the first time point (6.67%). Regarding the biochemical and antioxidant parameters, the highest SOD activity in kidneys and livers was observed for Group III (183.36 and 175.26 units/g protein, respectively). Similarly, groups II and III achieved the best CAT results for livers (7840180 and 7526357 nmol/min/g protein, respectively), while Group II significantly increased the GSH-Px activity (kidneys = 205.35 units/g protein; livers = 133.60 units/g protein and serum = 64.57 units/mL protein). Regarding the MDA levels, groups III exhibited the lowest levels (44968 and 54566 nmol/g protein for livers and kidneys, respectively). These results highlight the efficacy of A. arguta leaves extract as nutraceutical ingredient. Further studies should be performed to identify the metabolites responsible for these activities.

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[S&BE 3]

Development of functional cookies enriched with chestnut shells extract - Impact of gastrointestinal digestion on phenolic composition, bioaccessibility, and bioactivity

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Chestnut (Castanea sativa) shells (CS) are an appealing source of antioxidant compounds embracing interesting pro-healthy effects [1]. Recent studies have validated a nutraceutical extract from CS, recovered by an eco-friendly technology namely Subcritical Water Extraction (SWE), by in-vitro and in- vivo assays [2,3]. Nevertheless, the incorporation of this extract into a functional food with potential health benefits remains unexplored.
The purpose of this study was to develop and characterize functional cookies enriched with the previously validated extract, exploring the effects of the in-vitro gastrointestinal digestion in the total phenolic and flavonoid contents (TPC and TFC, respectively), antioxidant/antiradical properties, bioaccessibility, and phenolic profile of the CS extract-enriched cookies. Multivariate data analysis was performed to outline the differences between the digested samples and cookies.
The outcomes revealed an interesting phenolic profile and bioactivity of the functional cookies fortified with the CS extract. Using an in-vitro digestion model, it was observed that a higher concentration of polyphenols was retained in the intestinal fraction, which is possibly correlated to its better antioxidant/antiradical activity. The intestinal-digested cookies also showed higher radicals scavenging proficiency, particularly against HOCl and ONOO-. The phenolics recovery enhanced in the following order: oral<gastric<intestinal phases, achieving 93.50% of the maximum bioaccessibility. The phenolic composition was characterized by phenolic acids, hydrolyzable tannins, flavonoids, and one alkaloid. Regardless the identical phenolic composition rich in phenolic acids, changes in their concentrations were attested between digested and undigested cookies. A protective effect of cookie matrix on the phenolic compounds was unveiled with slow release during digestion. The multivariate analysis predicted the outstanding contribution of polyphenols to the bioactivity of cookies after in-vitro digestion. This study highlighted the valorisation of CS as nutraceutical ingredient of functional cookies, proving its efficacy after in-vitro simulated digestion.

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[S&BE 4]

Potential therapeutic application of chestnut shells in oral mucositis treatment: Green extraction of polyphenols

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Oral mucositis (OM), a common side effect of oncological treatment, is an inflammatory condition characterized by painful ulcerations, difficulty swallowing and speaking, and increased risk of infection, which can compromise the primary treatment outcome [1]. The use of natural antioxidants to inhibit theredox imbalance responsible for OM development has emerged as an interesting approach for OM prevention and treatment. This study aims to explore chestnut (Castana sativa) shells (by-products) aspotential active ingredient against OM [2]. To recover antioxidants using sustainable technology, chestnut shells were extracted using Subcritical Water Extraction (SWE) at different temperatures (110-180 °C). The extracts were evaluated against microorganisms present in the oral cavity as well as in human oral cell lines (TR146 and HSC3). The optimal extraction temperature was 110 °C, exhibiting thehighest phenolic content and the best antioxidant/antiradical activities and scavenging efficiencies against HOCl (IC_{50} = 4.47 µg/mL) and ROO• (0.73 µmol TE/mg DW). High concentrations of phenolicacids (e.g., gallic and protocatechuic acid) and flavanoids (catechin, epicatechin and rutin) characterizedthe phenolic profile. Antimicrobial activity against several microorganisms present in the oral cavity during OM, such as Streptococcus, Staphylococcus, Enterococcus, and Escherichia, was also demonstrated. The effects on HSC3 and TR146 cell lines revealed that the extract prepared at 110 °C showed the lowest IC_{50} (1325.03 and 468.15 µg/mL, respectively). Therefore, this extract was selectedfor encapsulation and incorporation into an oral film as buccal drug delivery system. Methocel 1000 demonstrated ideal physicochemical properties from different set of polymers, such as thickness, mass, content uniformity, dissolution time in artificial saliva, superficial pH, folding, tensile strength, and elongation.

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Ultrasound-Assisted Extraction of goji berries: Bioactive composition and pro-healthy properties

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Lycium barbarum fruit, also known as goji berries, have been used for thousands of years in herbal medicine [1]. The plant grows mostly in the Asian continent [1, 2], presenting a high nutritional value and rich bioactive composition, particularly in phenolic compounds, vitamins, sugars, fibres, organic acids, minerals, and fat acids [3]. The potential health properties of goji berries, such as antioxidant, antimicrobial, immunomodulatory and anti-inflammatory effects, have been highlighted in recent years [1, 4, 5], allowing to classify the fruit as a "superfruit" [2, 6] with interest for different industries, such as food, nutraceutical or cosmetic.

This study aims to evaluate the optimal ultrasound-assisted extraction (UAE) conditions of antioxidants polyphenols from *L. barbarum* berries using a mathematical model, namely Response Surface Methodology (RSM). The effects of solid:liquid ratio (2.5–10.0% w/v), time (20–60 min), and intensity (30–70 W/m^2) on the total phenolic content (TPC) and antioxidant/ antiradical activities were investigated. The optimal UAE conditions were achieved using a solid:liquid ratio of 8.75% (w/v) and an ultrasonic intensity of 59 W/m^2 for 56.21 min, showing a high TPC (23.87 mg of gallic acid equivalents (GAE)/g dw) and antioxidant/antiradical activity (15.15 mg of ascorbic acid equivalents (AAE)/g dw for ABTS assay; 10.25 mg of trolox equivalents (TE)/g dw for DPPH assay; and 105.97 µmol of ferrous sulfate equivalents (FSE)/g dw for ferric reducing antioxidant power (FRAP)). The *in vitro* cell assays demonstrated that the goji berries extract did not affect the viability of Caco-2 and HT29-MTX cell lines in concentrations lower than 100 µg/mL, presenting results between 80% and 100%. These results emphasize the great potential of goji berries extracted by UAE for food industry application. Further studies regarding *in vitro* intestinal permeation should be performed in order to understand the bioactive compounds responsible for the *in vivo* effects.

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[S&BE 6]

Salt replacement by *Salicornia ramosissima* to reduce biogenic amines formation in fish

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Salicornia ramosissima is frequently used as a salt replacer due to its high salt content. Its richness in phenolics, organic acids, minerals, and fiber make it an interesting natural preservative. The impact on biogenic amines formation from replace salt by seasoning fish products with salicornia has not been explored. Biogenic amines (BA), including histamine (HIM), cadaverine (CAD), putrescine (PUT), 2-phenylethylamine (2-FEN), tryptamine (TRI) and tyramine (TIR) are a group of undesirable compounds that can arise in foods, such as fish during its shelf-life. Their consumption by susceptible individuals can lead to poisoning, especially histamine [1]. Therefore, reduction of their formation during shelf-life of fish products is of major relevance. The objective of this work was to explore the possibility of using *Salicornia ramosissima* in the formulation of fish hamburgers (salmon and hake) to replace salt seasoning (1%) and protect against biogenic amines formation.
Biogenic amines were analysed by reverse phase HPLC with fluorescence detector, after acid extraction and derivatization with dansyl chloride. On the day of fish acquisition (day 0), salmon presented 5.0, 1.7 and 1.2 mg/kg of HIM, PUT and CAD, respectively and hake presented 8.3, 0.6 and 0.3 mg/kg of HIM, PUT and CAD, respectively. In both fishes tryptamine was detected but not quantified (< 0.487 mg/kg) and 2-FEN and TIR were not detected. On day 2, a considerable increase of all BA was observed in salmon burgers (∑BA control salmon = 257 mg/kg). The salicornia seasoning led to a mild inhibition of BA formation in those hamburgers, without statistical significance (∑BA 226 mg/kg). On days 5 and 8, a considerable increase in BA continued to be observed. However in hake, on day 2, the increase in BA was negligible (∑BA control: 12.6 mg/kg), and no significant effects could be observed by the addition of salicornia (7.9 mg/kg). However, on day 5 (∑BA control = 56.0 mg/kg), a significant reduction ($p < 0.05$) of the total BA content was observed in the hamburgers seasoned with salicornia (∑BA = 42.6 mg/kg), especially by inhibition of cadaverine formation (38.2 mg/kg vs 18.0 mg/kg). By day 8, the ∑BA of the control burgers increased to 117 mg/kg, whereas in burgers with salicornia BA content was similar to that observed on day 5 (42.9 mg/kg). Salicornia salt was not able to significantly prevent BA formation in salmon samples. Contrary to hake burgers, in which a strong inhibition of total BA was observed on days 5 (~ 50%) and 8 (~ 80%), this effect being greater than that observed by the addition of salt (~ 20% on day 8), and especially observed in cadaverine.

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[S&BE 7]

Reduce acrylamide formation in biscuits by fiber enrich the receipt

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Acrylamide (AA) is a food chain contaminant formed in starchy food products processed at high temperatures (frying, baking, roasting or industrial processing, at +120 °C) and low moisture. The most important food groups contributing to acrylamide *exposure* are fried potato products, coffee, biscuits, crackers, crisp bread and soft bread. Acrylamide is formed when the amino acid asparagine reacts with reducing sugars, such as glucose and fructose, being the main chemical processes that occur known as the Maillard Reaction, the same reaction that 'browns' food and gives distinctive flavour. EFSA considered AA as a matter of public health concern and AA levels across the EU have been subject to a benchmarking system since 2018 to force effective reduction of its content in food.
The aim of this work is the search for promising ingredients that can reduce AA formation on biscuits and improve their health benefits through fibre enrichment. Apple pomace, a by-product from apple juice industry (kindly provided by Sumol+Compal®) lyophilised and washed to remove free sugars was used as a source of fibre and partially replaced wheat flour. Positive control biscuits were prepared without fibre enrichment and without asparaginase addition, while negative control biscuits were prepared using asparaginase, as usually done by the industry. AA content in baked biscuits was assessed by gas chromatography (GC) coupled to mass spectrometry (MS) after solid-liquid extraction and derivatization with xanthydrol.
Analyses revealed that biscuits with apple pomace are richer in fibre (contain around 20%) when compared with positive control without asparaginase (5% fibre) and presents less quantity of AA (132.3±8.34 μg/kg *vs* 215.3±11.3 μg/kg). The reduction of AA content due to fibre addition is notorious, but alone did not reach the reduction obtained in negative control biscuits prepared with asparaginase (65.02±11.3 μg/kg). In the future studies, both strategies in combination will be used, to reduce the amount of asparaginase needed, and simultaneously increase the fibre content. Our results provide useful information for the food industry, as a new strategy to mitigate AA content in food through the bio-circular economy, using a cheap ingredient, the by-product from apple juice industry. In addition, biscuits with apple pomace are "high-fibre" food, being nutritionally improved in relation to the traditional recipe.

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[S&BE 8]

The Kombucha Secret - Scoby's Microbiome Study and ProbioticBacteria Identification

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Kombucha is a non-alcoholic tea-based fermented drink produced by a Symbiotic Culture of Bacteria and Yeast (SCOBY). In recent years, popularity increased due to nutritional and health benefits, pleasant flavour and sparkling due to the release of carbon dioxide produced during fermentation[1]. As a result, many studies have characterized the microbial community present in the the beverage, but exploratory studies of the microbiota in scoby are still scarce.

Therefore, DNA was extracted and the microbiota (Bacterial and Fungi) in scoby and kombucha was characterized. For this, a metabarcoding assay by long-read Next Generation Sequencing (Oxford nanopore Technologies) was performed in order to evaluate the community of microorganisms (bacteria, yeasts and fungi) present in the starter culture (scooby) and the resulting kombucha. We also explore the presence of probiotic microorganisms that can have positive health effects on the consumers of this fermented drink.

This study is extremely relevant to fully understand how fermentation evolves and how standardized starter cultures can be developed for future Kombuchas. This is the first step to characterize, explore and to understand how microbes interact and evolve during the production of this beverage.

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[S&BE 9]

In-situ enzymatic conversion of sucrose into prebiotic fructooligosaccharides for the development of a functional strawberry preparation

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The increased search for reduced-sugar and healthier food products has driven the growth of the functional food market [1]. This opened space for the development of *novel* functional products. Frulact SA, a partner in this project, is specialized in the development and production of fruit-based preparations, which are mainly utilized in the dairy industry for incorporation in flavored yogurts. Its market is expected to increase at a compound annual growth rate of 6.1% until 2030 [2]. However, despite being rich in nutrients, these preparations have a high amount of caloric added sugar. To reduce this sugar in a strawberry preparation, we herein propose an *in-situ* enzymatic conversion of its sucrose content into prebiotic fructooligosaccharides (FOS) [3,4].

Two commercial enzymatic complexes were evaluated for the *in-situ* synthesis of FOS. At optimal conditions (60 °C and pH 5.0), Pectinex® Ultra SP-L yielded 0.57 ± 0.01 $g_{FOS}/g_{ini.sucrose}$ after 7 h reaction and Viscozyme® L, 0.66 ± 0.00 $g_{FOS}/g_{ini.sucrose}$ after 5 h. The resultant strawberry preparations contained more than 50% (w/w) of FOS in total carbohydrates. Also, more than 80% of the original sucrose content was reduced, diminishing its caloric value by 31%. The data show that consumption of dairy products containing 10% of the developed prebiotic preparation would result in the ingestion of >2.5 grams of FOS per 100 mL of product. The prebiotic preparation showed also to resist the harsh conditions of the gastrointestinal tract since more than 90% of FOS were not hydrolyzed during digestion. The conversion of sucrose into FOS changed some physicochemical and textural attributes of the original product (*i.e.*, sweetness, color, viscosity, consistency), yet those can be easily adjusted.

The *in-situ* technological approach here developed shown great potential as an innovative strategy for the development of low-sugar and low-calorie prebiotic food.

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[S&BE 10]

Designing novel sweeteners for sugar reduction in foods

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Over the last years, industries have been seeking for solutions to replace sugar in their food products. However, the current options usually result in the loss of texture and changes in the flavour profile, perceived by the consumer as negative.[1] Besides sucrose, lactose, fructose and glucose, there are other sugars able to provide sweetness and texture while simultaneously contributing to improve the dietary fibre content without caloric contribution. These include fructooligosaccharides (FOS), which 1)→Fruf-(β2→ backbone structure is unable to be metabolized by human digestive enzymes.[2] FOS can be produced from inulin depolymerization, turning inulin rich foods a relevant resource for the design of non-caloric carbohydrate-based sweeteners. In this context, this work aimed to design novel FOS-based sweeteners by taking advantage of yacon agri-food wastes, an inulin-rich tuberous root[3] efficiently cropped in Portugal. To achieve this, the inulin-rich juice extracted from yacon was acidified and boiled to promote inulin depolymerization to FOS while performing its concentration through water evaporation until 73 °Brix.

The acidification of yacon juice boosted the thermal depolymerization of inulins, yielding syrups composed of different FOS structures as 1-kestose, nystose and 1,1,1-kestopentaose. The increase in FOS proportion promoted syrups level of sweetness, ranging from 40-50% to the sweetening power of sucrose. However, to provide the same sweetness intensity, the developed syrups only provide 75% of sucrose caloric contribution. The extent of inulin depolymerization to FOS was also found to modulate syrups stickiness and stringiness, reaching comparable texture properties of commercially available alternatives when FOS accounted up 30% of syrups carbohydrate composition. In this way, yacon agri-food wastes comprise a relevant resource able to produce new sweet-tasting ingredients with potential to surpass the current limitations of sucrose alternatives, and even new product development following tailored sugar reduction in foods.

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[S&BE 11]

New insights into molecular interactions between phenolic compounds and dietary (egg and yeast) proteins

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Phenolic compounds (PCs) are bioactive compounds which can be described as promising tools to reduce the use of synthetic additives[1]. Besides the health-promoting effects[2], PCs have been described as able to modulate the main organoleptic characteristics of plant-derived foods[3,4] while theycan be also used as antioxidant or antimicrobial agents[5]. Furthermore, the natural ability of phenolic compounds (PCs) to bind to proteins was studied to enhance the emulsifying capacity of proteins.

In this study, the molecular perspective of the use of PCs to enhance the emulsifying capacity of proteinshas been studied in a yeast protein extract (YPE)-based mayonnaise in comparison with the traditionalegg derived mayonnaise[6]. Thus, the molecular mechanisms of the interaction between egg or YPE protein models and PCs (blueberry extract, cynidin-3-O-glucoside, green tea extract and epigallocatechin gallate were unravelled by fluorescence quenching. The molecular binding models were studied at pH 7.4 (biological conditions) and at pH 3.5 (mayonnaise conditions) and at different temperatures simulating the storage conditions.

The Stern-Volmer plots show a different behaviour for the interaction between the PCs tested with the YPE compared to the egg proteins. In general, the Stern-Volmer plots for egg model were calculated byusing the Stern-Volmer equation to determine the molecular affinity constants (K_{SV}) by linear regression.For YPE interactions the Stern-Volmer plots presented a downward-curve towards the x-axis and the Ka was calculated applied the Stern-Volmer modified equation. A generally trend to higher constant affinity was observed in YPE model when compared to egg proteins. The results obtained within this study showed the potential of PC to enhance the emulsifying capacity of YPE, which can conquer the food industry in response to the consumer demand for clean labelling and potentially health-beneficial foods. However, future studies are required to understand the structure/activity relationships and main dose/response behaviours.

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[S&BE 12]

Iodine profile in foods consumed in vegetarian diets

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Vegetarian diets have gained popularity in the last decade, and it has been found that increased consumption of plant-based foods has numerous health benefits [1]. The number of vegetarians has quadrupled in the last ten years, currently representing around 9% of the population residing in Portugal [2,3]. Therefore, the nutritional study of the foods most consumed by the vegetarian population becomes important. Iodine is an essential nutrient for the synthesis of thyroid hormones. If the supply of iodine is compromised, the consequences may be hypothyroidism, goitre or the formation of nodules. When nutritional deficits occur in lactating or pregnant women, irreversible repercussions on babies or foetuses may occur[1,4,5]. The objective of this study was to evaluate the iodine content, by inductively coupled plasma mass spectrometry (ICP-MS), in 37 foods most consumed by the vegetarian population. Food selection was based on the National Food and Physical Activity Survey. Among the analysed foods, Nori seaweeds have the highest levels of iodine, 6511 ± 321 µg/100 g, followed by dairy products, namely cheeses (between 53.4 ± 0.4 and 28.0 ± 0.9 µg/100 g) and yoghurts (between 17.2 ± 0.2 and 14.4 ± 0.1 µg /100 g). When resorting only to food, the vegetarian and vegan population may have difficulty to achieve the daily iodine requirement (150 µg/day). In the case of pregnant and lactating vegetarians or vegans, nutritional deficiency can be a more worrying situation, taking into account that it can cause neurodevelopmental changes in the foetuses and baby. It is necessary to consider alternatives that can alleviate the nutritional deficits of iodine and prevent possible consequences on health. These may include using iodised salt, crop fortification, food fortification or supplementation.

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Metagenomic study of bacterial resistance and its epidemic potential in estuarine aquaculture farms in Portugal

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The growth of aquaculture farms to meet the increasing demand for fresh fish can lead to the overuse of antibiotics to control disease and promote growth. Moreover, it is recognized that antibiotic-resistance genes can spread in microbial communities, between bacteria, either pathogenic or commensal, by mobile genetic elements. These genetic traits can flow between aquatic environments, such as aquaculture farms, and the human environment, which affects human and animal health and the ecosystem [1]. Thus, when transmitted to the human microbiome, resistance genes pose a risk to human health by compromising the eventual treatment of infections with antimicrobial therapy. This study aimed to define the resistance profile of aquaculture farms and their potential risk of spreading.

We generated a collection of metagenomes from sediment extensive oyster and semi-intensive gilthead bream cultures, located in areas from north to south of Portugal: Lima and Sado River estuaries and in the Aveiro Lagoon [2] and performed comparative metagenomic analysis.

The computation of the diversity of genes conferring antibiotic resistance revealed that they fall into two significantly different groups according to the type of farm. In all geographic locations under study, the greatest diversity of resistance is found in genes conferring resistance to the macrolide, tetracycline and oxazolidinone classes of antibiotics, all of which are medically important in both human and animal therapies, as well as resistance to disinfectants. We also found that the number of mobile genetic elements correlated with the number of antibiotic resistance traits in oyster farms, suggesting that antibiotic resistance could be easily mobilized between bacterial genomes and microbiomes [3]. These particular resistances could compromise effective treatment of infections in humans if transmitted through the food chain.

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Production of a safe and natural red extract by *Monascus purpureus* fungus for food applications

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The colour of food is one of the most important factors which is immediately perceived by human senses.It represents the freshness and safety of foods. In recent years, the food industry has focused on producing natural extracts to overcome the use of synthetic colorants which are potentially harmful to human health and the environment. Among natural extracts, those from microbial sources are of great promise.
Monascus purpureus extracts and pigments have been used for manufacturing food colorants (e.g. redrice) and fermented foods in East Asia[1,2]. Among the several pigments produced by M. purpureus, the olgoketides, rubropunctamine and monascorubramine are the ones responsible for the red pigments[3].
The main aims of this work were i) to produce M. purpureus red extracts from a well-established method;
ii) to determine the encapsulation conditions for extracts stabilization; and iii) to evaluate extracts cytotoxicity in an in vivo model.
For red extracts production, one plug of the fresh culture was grown for 20 days at 30 ºC in Potato dextrose medium supplemented with glucose (20 g/L), peptone (3 g/L) and Fe (0.14g/L) at pH 5, according to the Mousa et al. [4]. The pigments were precipitated by adding two volumes of 95% (v/v) and all the solvent was evaporated using a rotary evaporator. To stabilize the colour the freeze-dried extracts were microencapsulated using gum arabic and maltodextrin as encapsulating agents. Colour variation tests revealed that maltodextrin encapsulant strategy was more efficient in the stabilization ofthe red colour than the use of arabic gum encapsulant. The extract does not show toxicity when the Galleria mellonella in vivo model was used.
Overall, these results open the possibility of using the red extracts produced by the M. purpureus fungusas a safe and stable natural pigment for food applications, namely in processed meat products.

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[S&BE 15]

Phenolic composition and antioxidant capacity of *Sarcocornia*

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Extremophile plants comprise species capable of growing in the most challenging environments, such as those dominated by ice in Antarctica, severe drought in the Sahara desert, or high levels of salinity and ultraviolet radiation in estuaries. The species of the latter group that thrive in environments of high salinity are considered a subtype of extremophiles, known as halophytes. In response to conditions of extreme saline stress, these species have developed highly specialised morphological and physiological adaptations, among which stands out the synthesis and accumulation of phenolic compounds [1]. The present work reports the phenolic profile and antioxidant capacity of *Sarcocornia perennis alpini* from the Mondego estuary. The total content of phenolic compounds (TPC) was determined by the Folin-Ciocalteu method, the total content of flavonoids (TCF) by the aluminium chloride assay, and the phenolic profile by UHPLC-ESI-PDA-MS/MS. Antioxidant capacity was assessed by both the DPPH radical assay and the β-carotene bleaching test. *S. perennis* exhibited a TPC of 38.2 mg of gallic acid equivalents/g extract dry weight (dw), a TFC of 99.3 mg of epicatechin equivalents/g extract dw, and a considerable antioxidant capacity, namely 11.0 mg of trolox equivalents/g extract dw by the DPPH assay, and by the β-carotene test, the coefficient of antioxidant activity was 1403. All in all, relevant amounts of phenolic acids and flavonoids were detected, which is in line with the observed antioxidant capacity. Hence, *Sarcocornia* can be considered an interesting source of phenolics with potential for application in the food and pharmaceutical sectors.

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[S&BE 16]

Green tea extract for application in active food packaging: balancing antioxidant properties and colour

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Antioxidants are characterized as molecules capable of preventing or delaying substrate oxidation [1]. In the European Union, the use of synthetic antioxidants in the food industry has been increasingly restricted, due to the need for their approval as food additives, according to Regulation (EC) No. 1129/2011 of the European Commission. In this way, there is a growth in research and application of natural antioxidants as potential substitutes for synthetic antioxidants [2], namely, the use of plant extracts [3].
Several researchers claim that the incorporation of green tea extract in food products is very promising in such a way that it extends the shelf-life and benefits the preservation of various foods. Green tea has several advantages, namely its, antimicrobial, anticancer, antidiabetic, antimutagenic, antihypertensive, cardiovascular disease prevention, oral health protection, and prevention of hypocholesterolemic disease [4-6].
In this work, several extracts of green tea were produced with the incorporation of different brighteners namely, sodium bicarbonate, eggshell (calcium carbonate), flavedo, and albedo of citrus and active charcoal, with the purpose of lightening the colour of active packaging prepared with green tea extract. All extracts obtained were submitted to the evaluation of antioxidant capacity by applying methods based on electron transfer (ET), which involves an oxidizing agent and antioxidant compounds [7]. The ET-based methods used were the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical inhibition system [8] and the bleaching test of β-carotene [9].
The results of this study are promising since it was possible to prepare food-grade green tea extracts with high antioxidant capacity and lighter colour. These extracts will allow the preparation of active films intended for food packaging with a lighter colour, without affecting their antioxidant effectiveness.

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[S&BE 17]

Is it possible to lighten the colour of *Camellia sinensis* (green tea) food-grade extracts without compromising their content in total phenolics and flavonoids?

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Antioxidants are present in food products, where they protect macromolecules by counteracting oxidation processes, for example by stopping the propagation phase or by inhibiting lipids' contact with precursors of lipid oxidation [1].
Synthetic antioxidants are the most used compounds due to their low cost and good stability; however, they are associated with the promotion of carcinogenesis in the organism, toxicity, or other negative health effects [2]. Due to this fact, it is increasingly necessary to replace synthetic antioxidants with natural antioxidants, and some plants such as green tea (Camellia sinensis L.) can be used to prepare food-grade extracts with high antioxidant capacity. In fact, lately, there is a growing interest in the application of green tea extract in foods as a natural antioxidant [3].
About two hundred compounds are present in green tea, namely, phenolic compounds, inorganic elements, methylxanthines, and others [4]. The phenolic compounds present in green tea are mainly catechins and their derivatives, which have bioactive properties [5]. The presence of high concentrations of phenolic compounds is related to high antioxidant capacity [6].
In this work, green tea extracts were produced by solid-liquid extraction with ethanol. Moreover, different bleaches were tested in order to evaluate their effect on the final colour. With the objective of lightening the colour of the final extracts, several brighteners were tested, such as, sodium bicarbonate, eggshell, peel/white part of citrus, and active charcoal. The determination of total phenolic compounds through the Folin-Ciocalteu method [7] and the determination of total flavonoids [8] was performed in all the extracts and it was possible to conclude that some of the tested bleachers do not compromise the total content of phenolics and flavonoids.
In future work, the lighter green tea extracts obtained will be ~~properly~~ used for active food biopackaging, with the aim of ensuring greater quality, food preservation, increasing shelf-life extension, and reducing environmental impact.

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[S&BE 18]

Analytical Strategy to Evaluate Stress in laying hens from Intensive Eggs Production

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In intensive animal production for food industry, one of the major problems is related with the animal welfare and consumers are increasingly aware of this problem. Biosecurity measures tools can help in the control and improvement of healthy animal farming and the Sustainable Development Goals (SDG), the SDG 12 highlights the need for a more responsible consumption and production of food and special attention should be provided to animal welfare.

Egg is an animal food product largely consumed worldwide. The laying hens and the intensive egg production systems can be severe for poultry, including restricted spaces with shared drinkers and feeders together with weak nutritional and hygiene conditions, leading to a stressful environment for the animals. An indicator of stress in poultry is the occurrence and raising of corticosterone concentration that may be detected in animal tissues and eggs [1,2].

The main goal of the present work was to develop and validate an ultra-high performance liquid chromatography method coupled with a triple quadrupole mass spectrometry detector (UHPLC-MS/MS) to detect and quantify the levels of corticosterone in eggs. Several extraction procedures were tested and the final method was fully validated for the determination of corticosterone in full eggs. Validation, and acceptance criteria evaluation, was performed in accordance with the Commission Implementing Regulation (CIR) 808/2021 [3] in which the parameters assessed were: specificity, selectivity, linearity, precision, recovery and CCα.

The method presented can contribute to monitor the quality of laying eggs production and in helping to certify the final product, the egg.

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[S&BE 19]

Phytochemical evaluation, antioxidant, and anti-aging capacities of honey from *Pittosporum undulatum* Vent., naturalized in the Azores Archipelago

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Pittosporum undulatum Vent. is an invasive species non-indigenous scattered across all Azores's Islands and its control take to many costs and work to implement. In this sense, honey originated from this tree could be an answer to take profit from it [1,2]. This botanical source influence its organoleptic and pharmacological characteristics (antioxidant and anti-aging capacities) which are commonly associated and regulated by the chemical composition, including phenolic compounds, enhancing its commercial value and higher demand from the consumer [3].

This work aims to evaluate honey from *P. undulatum* Vent. from Azores by the determination of its phenolic content and biological capacities, such as antioxidant and anti-aging capacities, in order to increase its value and equates its possible applications in several industries (food, cosmetic, and/or pharmaceutical). This study intends to assess the phenolic content, antioxidant capacity, through ABTS•$^{+}$, DPPH•, and FRAP assays, and the anti-aging capacity, through elastase and tyrosinase inhibition capacity.

This study yields extremely favourable findings. In fact, for the total phenols content the values ranged from 20.82 to 112.13 mg of GA/100 g, from 10.25 to 103.26 mg of GA/100 g for *ortho*-diphenols, and from 2.94 to 40.96 mg of CAT/100 g for flavonoids content. Concerning the antioxidant capacity, the values ranged from 0.06 to 2.27 mmol Trolox/100g, from 0.04 to 0.45 mmol Trolox/100 g, and from 0.05 to 0.69 mmol Trolox/100 g, for DPPH, ABTS, and FRAP, respectively. Regarding the anti-aging capacity, the inhibition capacity of honey was higher in elastase enzyme (ranged from 37.52% to 45.88%) than in tyrosinase enzyme (4.36% to 9.37%) [1].

To our knowledge, we present the first study concerning the phytochemicals and biological properties of honey from *P. undulatum* Vent., enhancing the possible health benefits, valorising at the same time, a national food product, and its possibles applications in several industries.

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Prunus lusitanica L. fruits as factories of compounds with bioactive potential

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Prunus lusitanica L. (Portuguese laurel or 'azereiro') is a rare species that grows naturally in different places in Portugal and has an important role in ecosystems' ecological balance and sustainability. To date, only two research studies have been published regarding the chemical composition and bioactivities of this plant's leaf extracts.[1,2] Uncovering the phytochemical composition and antioxidant capacity of rare fruit species is essential to justify their health benefits and increase their value.[3]

Thus, the present study aims to qualitatively and quantitatively evaluate the phenolic profile of *Prunus lusitanica* L. fruits during a 4-year study and correlate it with the antioxidant capacity, focusing on its potential future application. To the best of our knowledge, this is the first study that addresses both the phenolic composition and the antioxidant capacity of *Prunus lusitanica* L. fruits.

Identification and quantification of phenolic compounds were performed through HPLC-PAD-ESI-MS/MS (high-performance liquid chromatography–photodiode array detection-electrospray ionization tandem mass spectrometry) and the antioxidant capacity through ABTS (2,2'-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1 picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) assays. Twenty-one hydroxycinnamic acids, two flavan-3-*ols*, two anthocyanins, two flavonols, and one secoiridoid were identified and quantified in the fruits. The antioxidant capacity values obtained ranged from 8.76 to 11.76 mmol TE (Trolox equivalents)/100 g fw (fresh weight)); 7.88 to 10.69 mmol TE/100 g fw and 5.18 to 8.17 mmol TE/100 g fw, for FRAP, ABTS, and DPPH respectively.

Although further studies are needed, the findings of this study indicate that *Prunus lusitanica* L. fruits are a promising source of natural phenolic compounds with antioxidant potential and are thus suitable for future applications.

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[S&BE 21]

Salt pan waters can be exploited as a source of functional ingredients

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Marine environments are the warehouse of a variety of novel bioactive compounds prone to be explored. Polysaccharides, that are excreted by marine organisms, are one of these compounds, particularly the sulfated ones. Sulfated polysaccharides have been proposed as bioactive agents, regarding their potential antiviral, anticoagulant, antioxidant, and immunomodulatory activities [1]. Along these functional properties, some sulfated polysaccharides have already been explored as food additives due to gelling, emulsification, and thickening behaviours [2]. The growing interest in sulfated polysaccharides has led the search for new sources. Seawater is an available source of carbohydrates, which can be naturally concentrated in salt pan waters due to its evaporation by wind and sunlight [3]. Therefore, in this study polymeric material and polysaccharides were analysed along salt production in the evaporation ponds and in the crystallizer water.

Along salt production, polymeric material of seawater (13 mg/L) was accumulated in the evaporation ponds (9-73 mg/L) and in the crystallizer (133-144 mg/L). This polymeric material was composed by 29% of sulfated polysaccharides, with 45 mol% of sulfate esters, 23 mol% of uronic acids, 12 mol% of galactose, and 1 to 6 mol% of glucose, mannose, xylose, fucose, rhamnose, arabinose, and ribose. The uronic acid pattern was analysed by high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) [4]. Galacturonic (57%) and glucuronic acids (43%) were identified, whereas guluronic, mannuronic, and iduronic acids, common in some marine polysaccharides, were not detected.

These results highlight salt pan waters as a source of highly sulphated polysaccharide worth exploring as functional ingredients for food and feed applications.

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[S&BE 22]

Alginate hydrolysates, promising clean label substitutes of phosphates in ham

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Phosphates, namely tripolyphosphate, are food additives used for the retention of water in several protein-rich products, enhancing texture, tenderness, succulence, and production yield in processed meats [1]. However, high values of phosphate intake have been associated to cardiovascular comorbidity, as well as chronic kidney disease, justifying the search for clean label substitutes [2]. Alginate is an anionic polysaccharide from brown seaweed constituted by D-mannuronic and L guluronic acids. Due to its anionic properties, soluble oligosaccharides and low molecular weight polysaccharides from alginate are potential clean label alternatives to tripolyphosphates in meat products, mimicking their size and charge. Therefore, in this study alginate was depolymerised by microwave assisted partial hydrolysis at 120 °C (MW120) and 150 °C (MW150), without uronic acid degradation. Furthermore, the MW150 hydrolysate contained a higher content in monosaccharides, oligosaccharides, and low molecular weight polysaccharides in comparison with the MW120 hydrolysate as observed by high-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) [3]. Due to its carbohydrate composition, MW150 hydrolysate was incorporated in the cooked ham formulation. For comparison purposes, hams were also prepared without the additive incorporation (negative control), with the addition of sodium tripolyphosphate (positive control), and with commercial clean label alternative additives (not polysaccharide based). All hams had higher moisture (72 – 79%) than the ham without phosphate (67 – 69%). Textural analysis showed that ham with clean label substitutes achieved less hardness (21 – 42%) than ham without phosphate (49 – 65%). Nevertheless, moisture determination and texture profile analysis of cooked hams showed that MW150 alginate addition possessed similar moisture and hardness in comparison with the cooked hams with the commercial additives. Colour analysis showed that in comparison with hams with phosphate (positive control) all other formulations showed perceived differences, being slightly darker. These results show that alginate hydrolysates are promising clean label substitutes of synthetic phosphates additives in cooked ham formulations.

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Coffee soluble fibers: study of cholesterol-lowering mechanisms and their bioactivity after fermentation

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Coffee soluble dietary fiber (DF), mostly composed by polysaccharides (galactomannans and arabinogalactans) and melanoidins, have shown cholesterol-lowering properties.[1] The mechanism by which these compounds decrease cholesterol are associated to their bile salt sequestration capacity, and/or mediated by their fermentation products, acetate and propionate.[1,2] While the former serves as the primary substrate for cholesterol synthesis, the latter has been shown to inhibit cholesterol synthesis in hepatic tissues. Their ratio is therefore an important biomarker aiding on the management of cardiovascular diseases development.[3] Additionally, fermentation also leads to the biotransformation of phenolic compounds with known antioxidant capacity.[4]

The aim of this work was to evaluate cholesterol solubility dependence on the presence of coffee and its DFs, using two different in vitro intestinal models, composed of 1) a bile salt and a cholesterol standard, and a more complex containing 2) a bile salt, egg yolk as cholesterol food source and pancreatine, The most effective DFs were submitted to an in vitro fermentation, evaluating the acetate:propionate ratio, the microbial-derived phenolic metabolites and bacterial growth.

Arabinogalactans and melanoidins decreased cholesterol bioaccessibility to about 50%. Regarding their effect on short-chain fatty acid production, arabinogalactans yielded the lowest acetate:propionate ratio. Concerning phenolic compounds, the dihydrocaffeic and dihydroferulic acids were the main colonic metabolites (48 h of fermentation) found. The former was observed in higher amounts in melanoidins, while the latter was higher in arabinogalactans fermented samples. As for the microbiota composition, both DFs had a positive effect on the growth of lactic acid bacteria, which have been associated with several health benefits.[5] As a whole, this study showed that coffee can be used as a novel source of antioxidant dietary fiber, with potential to affect cholesterol homeostasis through different pathways, aiding on the prevention of cardiovascular diseases and promoting wellness and healthy aging.

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Occurrence of mycotoxins in feed: what's the animal risk?

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Animal diet is a combination of several feed raw materials, mainly composed by maize silage and compound feed, with other ingredients also being used as feedstuffs, such as ensiled by-products[1]. Storage and pre-harvest conditions lead to possible mycotoxigenic contamination of individual components of feed, which contributes to the presence of multiple mycotoxins from different origins and, consequently, to the final total daily intake of mycotoxins by dairy animals[2,3]. Scarcity of studies comprising the single contribution of each component is a current scenario that needs further attention for proper implementation of strategies of mitigation[4].

This study was focused on the identification of regulated and emerging mycotoxins in animal feed and representative feed materials (grass and maize silage samples) from Portuguese dairy farms, through an analytical methodology by liquid chromatography coupled to sequential mass spectrometry (UHPLC-QTrap-MS/MS). In this sense, we intended to evaluate the occurrence of these fungal toxins, and to identify the main sources of this occurrence.

In relation to the overall occurrence data, regulated and emerging mycotoxins were identified in all sample, namely grass silage, maize silage and animal feed. A high contamination was found at the level of maize silage, with major contribution to the occurrence of mycotoxins in the final product. Overall, the highest percentage of positives (100.0%) was found for the mycotoxins FB1 and FB2 in maize silage; DON in feed; ENNB in all analyzed samples; BEA and TTX in maize silage and feed. Other mycotoxins were identified with occurrence percentages higher than 40%.

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[S&BE 25]

Regional apple and pear cultivars from Alcobaça region (Portugal): Comparison of the antioxidant properties of the edible part and by-products

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Food waste is commonly defined as “a decrease, at all stages of the food system from production to consumption, in mass and/or quality, of food that was originally intended for human consumption, regardless of the cause” [1]. To avoid food waste, we aimed to characterize the antioxidant capacity of regional cultivars of apples and pears, for which there are still scarce data. Moreover, different parts of the fruits (peels, seeds and mesocarp) were analysed individually in order to evaluate the potential of by-products to be used a valuable source of natural antioxidants [2], which are believed to be responsible for human health benefits such as anticancer and cardioprotective effects [3].
The objective of this work was to determine the antioxidant properties [4] and fructose content of both by-products (peels and seeds) and edible part of five different Portuguese apple cultivars (Pardo Lindo, Pêro de Borbela, Noiva, Pêro Coimbra and Repinau) and five pear cultivars (Bela-Feia, Torres Novas, Carapinheira Roxa, Lambe-os-Dedos and Amorim), produced in Alcobaça region (Alcobaça), in two consecutive years. The antioxidant properties were evaluated through antioxidant capacity tests (DPPH radical scavenging and β-carotene bleaching) and total phenolic content and total flavonoids assays. By-products showed better antioxidant capacity, than the edible part in all cultivars, but the fructose content was very similar in the three parts of the fruits. The results indicate that regional apples´ by-products presented greater potential than regional pears´ by-products. In this line they have potential to be used as a valuable source of antioxidant compounds, which can be further used, by food, cosmetics, food packaging or food supplements industries.

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[S&BE 26]

Comparison of the phenolics profile of regional and commercial cultivars of apples (*Malus domestica*) from Alcobaça region (Portugal)

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Phenolic compounds are believed to have antioxidant properties, and therefore many studies have been conducted in order to search for the presence of such compounds in food matrices and the effects that they cause [1, 2]. Some of the most common sources of phenolic compounds in human diet are fruit and fruit based products, particularly apples [3].
An Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) method was developed for the determination of individual phenolics in the pulp and by-products of regional and commercial cultivars of apples from Alcobaça region (Portugal). The analytical method was evaluated regarding linearity, limit of detection, limit of quantification and accuracy, showing its suitability for the quantification of phenolic compounds.
Epicatechin, quercetin-3-b-d-glucoside, quercetin, chlorogenic acid and 4-hydroxybenzoic acid were the main phenolic compounds found in all portions of apples, namely: peels, seeds, and pulp (mesocarp).
Apple peels presented the highest content of phenolic compounds. For example, in the Noiva variety, peels had the highest concentration of epicatechin (80.69 ± 0.59 µg/g), followed by seeds (16.36 ± 0.48 µg/g) and lower levels were found in the pulp (3.74 ± 0.08 µg/g).
Phloridzin, one of the most recognized apple polyphenols, was quantified in all apples, however, different cultivars have different levels of this phenolic compound. In peels, the level of phloridzin ranges between 10.8 µg/g in the Bonita variety to 0.57 mg/g in the Fujion variety.
Some cultivars of apples showed very high levels of phenolic compounds, associated with their putative benefits for Human Health. Moreover, their by-products showed great potential as source of natural antioxidants.

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[S&BE 27]

Towards Citron (*Citrus medica L.*) valorization: an ancient citrus with excellent antioxidant properties and appreciated aroma

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Citron (*Citrus medica* L.) is a large fragrant citrus fruit, that resembles a lemon. This fruit is known for having a very small amount of pulp, if any and a thick skin. Because of this, its use by the food industry is very limited - nowadays, it is mostly consumed candied with sugar or in the form of a drink called cedrata. The medicinal benefits of this fruit are related with its antibacterial properties and antioxidant capacity [1-2].

The objective of this work was to evaluate the antioxidant properties, as well as estimate the total phenolics content (TPC) andotal flavonoids content (TFC) of the citrus fruit, divided into pulp, seeds, albedo and two ripening stages of the flavedo (yellow and green). The DPPH-radical scavenging and β-carotene bleaching colorimetric assays were used for the determination of the antioxidant capacity of the fruit portions. TPC and TFC determination assays were carried out following the procedure of Barbosa et al. [3].

The results show that the higher DPPH inhibiting power (IP) was obtained by the pulp, registering an IP of 18.29 ± 0.12%, whereas in the yellow and green flavedo, for example, it was only 10.51 ± 0.35% and 7.53 ± 0.12%, respectively. The albedo portion presented the lowest IP, recording an IP of 3.81%. However, the total phenolics content assay reveals that the highest level of total phenolics was found on the flavedo portions (1198.79 ± 1.1 µg GAE/g fresh fruit in the yellow flavedo portion and 1010.26 ± 1.1 µg GAE/g fresh fruit in the green flavedo portion), followed by the seeds (713.60 ± 1.1 µg GAE/g fresh fruit). Albedo had the lower TPC among all the portions studied (713.60 µg GAE/g fresh fruit).

The study provides valuable information towards the valorization of this ancient fruit in regard to its antioxidant properties, highlighting that Citrus medica L. have potential to be used as an excellent source of natural antioxidants that can have an impact on the food industry as well as in other industries, like the cosmetics and fragrances industries, where the citrus aroma is very much appreciated.

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[S&BE 28]

Characterization by UHPLC-ToF-MS of phenolic compounds of citron (*Citrus medica*), an ancient species of the genus Citrus

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Citron (*Citrus medica*) is a large yellow citrus fruit with a thick peel and an intense citrus fragrance. Citron, an ancient species of the genus Citrus, is considered the original lemon from which many different cultivars have been developed. Although this fruit has been very popular for its medicinal properties, nowadays its production is small and limited to crystallized in bakeries [1,2].
This study aimed to characterize the phenolic compounds present in the peels (including flavedo and albedo), seeds, and pulp of citron to improve the valorization of this traditional citrus fruit. For that, phenolic compounds were extracted using a mixture of methanol and water (50:50) with 0.1% formic acid. Twenty-seven phenolic compounds, including phenolic acids and flavonoids, were evaluated in citron samples by Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS).
The results showed that pulp presented the highest level of eriocitrin (353.78 ± 16.98 µg/g) and hesperidin (128.58 ± 3.78 µg/g), common phenolic compounds of the citrus family. The o-coumaric acid was determined in all parts of the citron, with the highest level in green flavedo (23.21 ± 0.38 µg/g). Different contents were determined on yellow and green flavedo, being rutin the major phenolic in yellow flavedo (35.92 ± 2.52 µg/g) while sakuranetin was the major phenolic in green flavedo (45.99 ± 2.02 µg/g). Albedo had the highest number of phenolic compounds identified, where eriocitrin (110.29 ± 0.39 µg/g) was the most predominant. Seeds had lower levels of phenolic compounds.
In conclusion, the pulp and peel of this ancient citrus fruit contain phenolic compounds that are recognized for their antioxidant properties; therefore, the findings support the valorization of citron. Citron can be used in the development of functional foods, promoting Human Health, and delaying food oxidation. In addition, citron imparts citrus flavor, which is highly appreciated by consumers.

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Classification of apple and pear species from Alcobaça region (Portugal) and their cultivars with machine learning algorithms

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Principal Component Analysis (PCA) transforms the original variable into new ones called principal components (PC). These PC´s are calculated attributing a coefficient for each original variables proportional to their contribution into this transformation in order to maximize the variances of the first few components [1]. The main objective is to reduce the dimensionality, while keeping the contribution of all initial variables in order to provide a visual pattern recognition [2]. PCA biplot graphs with both scores and loadings provide information on the influence of each variable on a given sample. The hierarchical clustering was also employed in order to highlight the similarities among samples.

The following variables were determined through ethanolic extracts of apple and pear varieties from the Alcobaça region (Portugal) using spectrophotometric analysis: DPPH radical scavenging, β-carotene bleaching, total phenolic content, total flavonoid content, and fructose content. The results show that a very high correlation exists among the variables antioxidant capacity through DPPH, total phenolics content and total flavonoids, while the fructose content shows independent behavior in relation to the other ones. The graph of scores for the first two PCs, which explain 90% of variance, shows three different clusters with different apple and pear species. It can be concluded from these results that the fructose content allows to separate apples and pears while antioxidant capacity through DPPH, total phenolics content and total flavonoids content can be used to separate different cultivars of each fruit.

This study shows that multivariate analysis, with special focus on PCA, can be a valuable tool for the separation of different fruit species and their cultivars highlighting the similarities and differences among them.

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[S&BE 30]

Evaluation of the individual phenolic compounds of regional cultivars of pears (*Pyrus communis* L.) by Liquid Chromatography combined with High Resolution Mass Spectrometry

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Pear (*Pyrus communis L.*) is a fruit of great interest because their consumption offers nutritional as well as medicinal advantages [1]. The most well-known and studied properties associated with pears are antioxidant and anti-inflammatory properties [2] – these are associated with the presence of phenolic compounds, mainly in the peels but also, in smaller amounts, in its mesocarp (pulp) [3].

In this work, an Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) method was optimized and validated for the determination of individual phenolics in the pulp and by-products of fruits.

The main phenolic compounds found in the studied regional pear cultivars (6 cultivars from Alcobaça region) were catechin, epicatechin, caffeic acid, chlorogenic acid, and vanillic acid.

The pear by-products (peels and seeds) presented higher phenolic content than mesocarp, mainly peels samples. One example of this is the *Carapinheira Roxa* cultivar, wherein the peels had the highest concentration of chlorogenic acid (26.26 ± 0.65 µg/g) and lower levels were found in the seeds (3.11 ± 0.19 µg/g) and in the pulp (4.27 ± 0.03 µg/g).

Other very well recognized pear phenolics, were quantified in all the pear cultivars, at different levels. For example, in the peels portion, the level of epicatechin varied between 0.19 µg/g in the *Torres Novas* cultivar and 37.30 µg/g in the *Lambe-os-Dedos* cultivar. The studied regional pear cultivars, in particular their by-products, presented high levels of phenolic compounds, showing potential to be valorized.

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[S&BE 31]

Antioxidant properties of thirty commercial cultivars of apples from Alcobaça region (Portugal): edible portion *versus* by-products

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Alcobaça is a region located in the centre of Portugal known by its unique edaphoclimatic conditions – that make it the largest apple production region in the country. The "Maçã de Alcobaça" is one of the 1257 products registered as Protected Geographical Origin (PGI) by the eAmbrosia database of the EU [1]. This fruit is widely appreciated worldwide, not only because of the organoleptic properties, but also because of their antioxidant properties and association with lower risk of cardiovascular diseases [2-3].
The objective of this work was to determine the antioxidant properties as well as estimate the fructose content of 30 commercial cultivars produced in the Alcobaça region. The antioxidant properties were evaluated through antioxidant capacity tests (DPPH radical scavenging and β-carotene bleaching). Moreover total phenolic content and total flavonoids were also determined. The fructose content was assessed as per Ashwell [4].
Overall, the by-products of the commercial cultivars of apple presented a higher antioxidant capacity than the pulp. The *Fujion* cultivar, for instance, presented a antioxidant capacity of 76.4 µg Trolox equivalents (TE)/g of fresh fruit on the peels, comparatively to 53.7 µg TE/g of fresh fruit on the seeds and 22.1 µg TE/g of fresh fruit in the pulp.
These results show that the by-products of the studied commercial cultivars of the studied apples fruit can be used as a valuable source of natural antioxidants, avoiding their waste and guaranteeing their valorisation for instance, by food industry, in a circular economy concept.

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[S&BE 32]

Nanotechnology-based strategies for the development of improved nutraceuticals and functional foods

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Functional foods and nutraceuticals are currently attracting increasing interest as they can have a significant impact on the improvement of different aspects of human health [1]. Nevertheless, several issues may compromise the delivery and efficacy of their active ingredients namely, a limited water solubility, low stability in the harsh environments of the gastrointestinal tract, and low permeability into different body barriers. Nanotechnology is an attractive approach to counteract these limitations, as nanoparticles may encapsulate different functional ingredients, improving their oral bioavailability and potentiating their health benefits [2]. Lipid nanoparticles are particularly attractive types of nanoparticles for food industry applications, as they are composed of generally recognized as safe components, have high stability, and can be produced using low-cost methods, which facilitates their large-scale production.

Here lipid-based nanoparticles have been used for the encapsulation of different natural bioactive ingredients aiming at the development of nutraceuticals and functional foods with enhanced characteristics and efficacy. The lipid nanoparticles revealed adequate characteristics for oral administration, high encapsulation efficiencies, and storage stability. Moreover, a controlled release of the compounds was obtained in simulated gastric and intestinal fluids and nanoparticles were able to cross *in vitro* models of the intestinal barrier, demonstrating their suitability for oral administration [3-5].

Overall our data demonstrated the benefits of nanotechnology in improving the performance of different bioactive compounds and highlightted the potential of these strategies to develop novel effective nutraceuticals and functional foods.

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Growth of persistent and sporadic strains of *Listeria monocytogenes* in cow's milk

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The production and consumption of dairy products are increasing worldwide [1]. As the single most important raw material in dairy production, the quality of milk is central to the quality and safety of all dairy products [2]. Due to its highly nutritious nature, milk is an excellent growth medium for a wide range of microbes [3]. Microbial contamination of milk and dairy products along the value chain remains a daunting task for the dairy industry [4].
Many outbreaks and sporadic cases of listeriosis are related to dairy products, such as soft cheeses and unpasteurized milk. [5]
Foods in which Listeria monocytogenes can survive and/or grow are potential causes of listeriosis when the storage (temperature/time) or preparation guidelines are not followed. [6]
This study was conducted to assess the growth of *L. monocytogenes* previously characterized as persistent and sporadic in cow's raw milk under storage temperatures. For this purpose, two strains were selected and were inoculated in raw, thermized and pasteurized milk. Enumeration of L. monocytogenes was performed in ALOA at different time intervals. Samples were examined while they visually fit for consumption.
The present study demonstrates that *L. monocytogenes* can grow slowly in raw milk and that the natural microflora is a key factor on its grow. In thermized and pasteurized milk *L. monocytogenes* achieved higher numbers than raw milk. These findings highlight the importance of consumer education regarding appropriate raw milk storage and handling. Susceptible individuals, for whom even low-level of *L. monocytogenes* contamination can present a health risk, should avoid the consumption of raw milk without prior heating.

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[S&BE 34]

Enzymatic extracts from the Macroalgae *Fucus vesiculosus*: antioxidant activity and total phenolic content analysis

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The marine environment is full of organisms, extremely rich in bioactive compounds, and with tremendous potential for pharmaceutical, industrial, and biotechnological applications. This work investigates the antioxidant profile and total phenolic content of enzymatic extracts (recurring to one protease -alcalase®, and two cell wall degrading enzymes - cellulase® (carbohydrase) and - Viscozyme® (a multi-enzyme complex of carbohydrases) from the macroalgae Fucus vesiculosus (FV)(brown, Phaeophyceae) obtained from land-based fully controlled cultivation systems under the Integrated Multi-Trophic Aquaculture (IMTA) sustainable concept and provided by ALGAplus® (Aveiro,Portugal). Total phenolic content was determined by the Folin method. Antioxidant capacity was measured by three different complimentary in vitro assays: ABTS total antioxidant capacity, DPPH free radical, and oxygen radical absorbance capacity (ORAC)). Results are presented in **Table 1**.

Table 1. Total phenolic content and antioxidant capacity of F. vesiculosus enzymatic extracts performedwith alcalase, cellulase, or viscozyme.

	Total phenolic content mg Gallic acid Equiv/ g dry extract	**DPPH** mg Trolox Equiv/ g dry extract	**ABTS** mg Trolox Equiv/ g dry extract	**ORAC** mg Trolox Equiv/ g dry extract
FV alcalase	311.30 ± 9.98[a]	0.181 ± 0.004[a]	90.32 ± 2.30[a]	197.08 ± 15.82[a]
FV cellulase	291.97 ± 1.06[b]	0.214 ± 0.007[b]	87.84 ± 4.57[a]	339.65 ± 24.73[b]
FV viscozyme	229.22 ± 5.50[c]	0.182 ± 0.002[a]	69.12 ± 3.84[b]	223.33 ± 14.87[c]

a–d, in a column: different letters indicate significant differences (p<0.05) between enzymatic procedures.

Higher total phenolic content and total antioxidant capacity were obtained for the extraction with alcalase(FVa), whereas the cellulase extraction (FVc) registered superior results for DPPH free radicals and ORAC. Viscozyme extraction showed no additional value on phenolics and antioxidant composition in comparison with cellulase extraction. This study presents promising applications of enzyme-assisted extraction for the obtention of different compounds with antioxidant capacity from the brown macroalgae *F. vesiculosus*.

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[S&BE 35]

Microalgae for sustainable food systems: development of *Chlorella vulgaris*-enriched 3D snacks

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Continuous world population growth, expected to reach nearly 11 billion at the end of the century, can exacerbate the challenge of ensuring that future development is sustainable [1]. As a result of increasing world population, dramatic impacts on global climate and ecosystems are expected to restrain current food systems, pushing for food demand to increase on a global scale. To avoid overexploitation of resources, food production systems have turned to alternative and unexploited foods, such as microalgae [2]. Microalgae have been considered as a key alternative ingredient as: 1) they show potentially high growth rates and can grow in different environments and, 2) have been shown to be a potential ingredient for functional foods, while presenting health-promoting nutrients [3]. However, microalgae present strong, distinctive flavor that constraints efforts towards developing microalgae food products as consumers' sensory acceptance is often negatively influenced [4]. To boost acceptance of microalgae foods, 3D food printing technology was applied in the present work to produce *Chlorella vulgaris*-enriched snacks that present an attractive multi-layered texture. Levels of incorporation of *C. vulgaris* in snacks (2 – 18%) were set according to raw dough's printability. Health impact of baked snacks (170 °C, 15 min) was analyzed by assessing its nutritional composition, including mineral profile, *in vitro* antioxidant activity (FRAP, ABTS, DPPH assays), total phenolics (Folin-Ciocalteu), pigments (chlorophyl and carotenoids) and *in vitro* digestibility and bioaccessibility (Infogest model).

Results showed that higher concentrations of *C. vulgaris* positively impacted snacks' nutritional composition, as well as promoting an increase of the bioactive compounds such as total phenolic compounds, minerals, and pigments. Snacks enriched with the highest levels of *C. vulgaris* presented higher antioxidant activities and increased mineral bioaccessibility.

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[S&BE 36]

NAM-Aptamers-based approach to control Salmonellosis in the poultry industry

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Salmonellosis is considered one of the main bacterial infections in poultry sector having an important negative economic impact worldwide. Several measures have been implemented over the years by the competent authorities as an attempt to prevent and control salmonellosis. Prevention and control of Salmonella infections must be done by combining all available resources and covering the entire food production chain. Aptamer-based technologies are a promising and innovative approach not only to treat infection with different pathogenic microorganisms but also to prevent the infection process. Regarding salmonellosis, one way to do this is by blocking the adhesion, thereby preventing salmonella from the intestinal epithelial cells.

This project focuses on blocking the adhesion protein SipA from Salmonella using SipA-specific nucleic acid mimic (NAM)-aptamers.

The Apt17 that recognizes SipA protein was selected based on current literature. Bioinformatic tools were used to predict possible tertiary structures of the NAM-aptamers. The structure of the aptamer-SipA complex was also analysed and the contact residues between them were identified to improve the aptamer by replacing specific nucleotides with non-natural nucleotides. In addition to the full SipA protein also a specific domain of the protein that plays an important role in the entry of Salmonella into host epithelial cells was analysed. The KD constants were determined using a real-time PCR methodology.

The binding assays confirmed the binding capacity of the Apt17 both to the full SipA protein and to the specific C-terminal domain. The KD value was estimated directly from the experimental results through the binding curve using a non-linear regression analysis.

This work aims to develop the first aptamer-based solution able to prevent/control Salmonella infection in poultry industry, as an alternative approach to avoid the side effects associated with the use of antibiotics.

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[S&BE 37]

Analytical Assessment of Contaminants in Food

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The constant use of pharmaceutical drugs in intensive food producing animals can result in the presence of those compounds, as contaminants, in the food chain. This situation can lead to undesirable effects in human, animal, and environment health. Concerning the use of antimicrobials and the spread of antimicrobial resistant bacteria, there may be a link between this potentially dangerous scenario with the overuse of antibiotics in both human and animal medicine [1,2]. On the other hand, the evaluation of the occurrence of natural contaminants, such as natural toxins as mycotoxins, along the entire food production chain, is also important given their recognized link to cancer diseases.
To effectively safeguard consumers, antimicrobial residues in food must be controlled to guarantee that the introduction of animal products into the food chain does not jeopardize the quality of the final food items. Similarly, the presence of mycotoxins should be monitored in order to assess contamination levels and support effective steps to ensure consumer safety.

Advances in chemical analytical technologies have enabled the development of multi-detection and multi-class methodologies, providing for a better understanding of the presence of persistent natural and anthropogenic contaminants [3–5]. These approaches are centered on ultra-high-performance liquid chromatography in tandem with mass spectrometry equipment, namely UHPLC-QTRAP-MS/MS and UHPLC-TOF-MS. The major goal of the work developed is to ensure food safety, consumer health, industrial and commercial interests, as well as acquire data and tools for evaluating the impacts on the environment and animal health. The analytical strategies recently developed and presented here as Food Safety tools are focused on the determination of antimicrobials in animal food products and the incidence of mycotoxins in the food chain: from maize seed to milk consumption.

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[S&BE 38]

Survival of sporadic and persistent *Listeria monocytogenes* through acidic conditions simulating stomach digestion

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Listeria monocytogenes is a major human and animal foodborne pathogen that causes listeriosis, a disease with a high fatality rate (> 40%)[1]. Listeriosis mainly affects high-risk groups, including neonates, pregnant women, the elderly and immunocompromised individuals[2]. After consumption of contaminated food, *L. monocytogenes* hold the capacity to cross the protective intestinal and placentalepithelial barriers. The ubiquitous nature and ability to grow in harsh conditions make this pathogen difficult to control by the food industry[3]. Some strains may be repeatedly isolated over time in the sameplant for several months/years – persistent strains[4]. This represents a major challenge for the food sector as cross-contamination by the equipment and general food processing environment is one of the most important sources of food contamination. From a risk analysis perspective, it is also important to assess the virulence potential of these strains that represent a continual risk of contamination. This study aimed to evaluate the survival of persistent and sporadic Listeria monocytogenes strains under acidic conditions, simulating the stomach digestion, when incorporated into two food matrices.

In total, thirteen strains (seven persistent and six sporadic L. monocytogenes strains) from food and food-associated environment were screened to evaluate their capacity to survive in an acidic condition(HCl; pH 3.0) when incorporated in a meat preparation and soft cheese. It was observed that cells werereduced by approximately 0.3 and 0.6 log cycles when incorporated in meat and cheese, respectively. This demonstrates that meat has a higher protective effect than cheese, possibly due to its high-fat content and higher pH (pH 6.7 for meat pulp and pH 5.7 for cheese). Although preliminary, these resultsdemonstrated that there seems to be no correlation between sporadic and persistent strains in survivingacidic conditions (stomach digestion).

Further tests should be performed to evaluate the survival of these strains along the gastrointestinal tract and to further investigate the impact of these persistent- and sporadic-strains on the gut microbiota.

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[S&BE 39]

Production of low-fat mayonnaise by substitution of additives by "clean label" ingredients from food by-products

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Mayonnaise is a creamy and smooth sauce used as seasoning and consists in an oil-in-water (O/W) emulsion. Its recipe contains 65-80% fat that is crucial for its texture and appearance. When the fat quantity in mayonnaise is reduced, the oil droplets density decreases, affecting the viscosity of the sauce. Therefore, low-fat mayonnaise contains additives that increase the emulsion stability and improve the final texture[1]. The objective of the present work is to modify the recipe of a commercial light mayonnaise (25% fat) by replacing the emulsifiers (xanthan gum, modified starch, and egg yolk) with other ingredients, such as aquafaba, pine nut skin, and brewer's spent yeast (BSY). A model solution of 25% oil and 75% aqueous buffer at pH 3 was used to mimic the mayonnaise and to test the effects of each of the three ingredients separately at different concentrations. Emulsion formation, stability over time and separation of phases were used as criteria to evaluate each candidate. Pine nut skin and BSY alone failed in the emulsion formation. Aquafaba (60 mg/mL) alone promoted the emulsification, but it was not able to emulsify the whole volume of the sample. When increasing aquafaba concentration to 120 mg/mL, the whole model solution was emulsified successfully. Moreover, different combinations of the three ingredients were tested and the combination of aquafaba, pine nut skin and yeast all together gave the best result. Likely, proteins and polysaccharides contained in aquafaba are crucial for the formation of O/W emulsions[2], while the surfactants contained in the pine nut skin can be used as stabilizing agents and BSY as Pickering agents. This set of ingredients (aquafaba 120 mg/mL, pine nut peel 1.0 mg/mL and yeast 2.5 mg/mL) was then substituted in the original 25% fat mayonnaise recipe. They promoted the formation of emulsion in the whole volume, but after 48h phases started to separate and after 7 days 18% of the emulsification was lost. These results showed that the combination of aquafaba, pine nut peel and BSY constitutes a promising alternative to industrial emulsifiers.

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[S&BE 40]

Differentiation between persistent and sporadic Listeria monocytogenes through growth kinetics

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Listeria monocytogenes is a facultative anaerobic, Gram-positive foodborne pathogen that can cause severe illness in vulnerable populations such as the elderly, immunocompromised individuals and pregnant women. When exposed to stressful environments such as low temperature, low pH or high salinity, *L. monocytogenes* enter a stationary phase exhibiting significantly slower growth rates. Certainstrains are continuously detected in the food processing environments (FPEs), something possibly explained by the formation of cell subpopulations capable of withstanding adverse conditions, which may encompass the high salinity, low temperature, or low pH which are subjected to.

Given this prospect, we set out to evaluate if persistent *L. monocytogenes* isolates collected from cheese factories present better fitness regarding their growth kinetics, focusing on two growth parameters (μmáx and lag phase) when faced with frequent stresses found in these FPEs compared with transient strains.

A two-level three condition full factorial design was implemented, with 18 Lm strains being grownin culture media which presented several combinations of pH (7.0 and 6.0 adjusted with lactic acid), NaCl (2.5 and 8.0%), and temperature (11-30 ºC). From the 18 tested strains, we did not observe statistically meaningful differences between the persistent and transient L. monocytogenes groups.

In conclusion, although a better understanding of the growth kinetics of L. monocytogenes is crucial for controlling and preventing its spread in FPEs, given our data, persistence does not appear tobe linked with growth fitness of specific strains of this pathogen.

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[S&BE 41]

Milk as a source of building blocks for nanoplatforms with biological applications

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Nanotechnology is an emerging scientific field with a tremendous number of applications among manytechnologies and products. In particular, the use of nanoparticles as delivery systems for therapeutics or nutraceuticals has been vastly investigated and applied towards overcoming limitations such as poorbioavailability or hazardous side effects. However, these nanocarriers are associated with some issuesthemselves such as toxicity of their constituents, which has, in turn, brought attention to the use of natural and biocompatible components to improve their biosafety [1]. Milk is a colloidal suspension composed mainly of water, fat, proteins and lactose some of which are organized to form naturally occurring nanostructures. Furthermore, milk and its derivatives have been used for millennia in food andhealthcare, making it an interesting source for the development of nano-systems [2].

In our group, we produce nanoparticles using components extracted from bovine milk via simple and easily scalable methods. These nanoparticles can be tailored to fit specific needs both regarding the intended delivery site and/or the agent to be delivered. This work has focused on three different nanoparticle formulations: lipid nanoparticles produced using the milk fat layer (obtained by centrifugation of the whole milk), nanoparticles produced using milk casein (extracted from the defattedmilk) and milk exosomes (naturally occurring nanoparticles extracted from the defatted milk after casein removal). The developed nanoparticles have all shown promising properties for applications in nutraceutical delivery, each with their own specific advantages. Furthermore, the sequential procedureused to obtain each component also enables a reduced waste generation ensuring a generally more environmental-friendly production.

Tu summarize, these combined studies showed that milk can be used as a promising and efficient source with which to produced nanoparticles suitable for a wide variety of food and health applications.

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[S&BE 42]

Antioxidant Potential Assessment of Commercial Herbal Infusions and Tea

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Herbal infusions are popular beverages and have been recognized as an essential source of antioxidants with putative benefits for Human Health [1]. The infusions prepared from different species and different parts of plants (flowers, leaves and roots) are rich in phenolic acids and flavonoids [2]. These compounds have scavenging ability against free radicals, which are key factors for food preservation, protecting against oxidation.
This work aims to assess and compare the antioxidant capacity of seventeen commercial samples of dried herbal plants for infusions and tea. For that, DPPH radical scavenging activity assay and β-carotene bleaching assay were carried out [3]. Moreover, the determination of total phenolic content and total flavonoid content was performed [3]. All infusions were prepared following the same procedure.
All the herbal infusions presented a high polyphenolic content, which is a good indicator of antioxidant capacity. Nevertheless, yerba mate (Ilex paraguariensis L.), lemon balm (Melissa officinalis)), and peppermint (Mentha × piperita) were revealed to have the greatest antioxidant capacity through both DPPH radical scavenging and β-carotene bleaching assays and were richer in phenolic compounds. For example, yerba mate, showed a high antioxidant capacity by DPPH radical assay (642.63 ± 5.18 mg trolox equivalents/mL) and β-carotene bleaching assay (antioxidant activity coefficient, AAC = 522.11 ± 4.81). Also, this sample revealed a high TPC (569.38 ± 0.39 mg gallic acid equivalents/mL) and a high TFC (1031.10 ± 7.78 mg epicatechin equivalents/mL). On contrary, milk thistle (Silybum marianum) and ginger infusion (Zingiber officinale) presented the lowest antioxidant capacity.
In conclusion, from the studied plants, yerba mate is a good candidate to be used to prepare food grade extracts with antioxidant properties. These extracts can be further used to replace synthetic antioxidant food additives or to be incorporated in polymers or biopolymers to obtain active food packaging. Active packaging with antioxidant capacity is useful to prevent lipid oxidation and extend the shelf-life of food products with high lipid content, being a more sustainable approach for the future of food packaging.

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[S&BE 43]

Mushroom as hypocholesterolemic functional ingredients

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Since ancient times, mushrooms have been increasingly appreciated in the traditional cuisine due to their nutritional characteristics. Mushrooms are considered as functional foods, since they present in their chemical composition several compounds, such as polysaccharides, proteins, sterols and statins [1, 2], which have bioactive properties, such as hypocholesterolemic properties [3, 4].

In this work, soluble fiber from four Portuguese endogenous mushroom species with gastronomic importance – *Hydnum spp*, *Lactarius spp*, *Macrolepiota spp* and *Tricholoma spp*, and a medicinal mushroom species – *Ganoderma spp* were extracted with hot water. The aqueous extracts from all mushroom species presented proteins (8 – 44 %) and polysaccharides (26 – 78%). The hypocholesterolemic effects of aqueous extracts of *Ganoderma spp* were addressed by following bile salts sequestration and cholesterol accessibility, however the high protein content in these extracts seemed to affect their emulsification behavior, leading to an increase on cholesterol accessibility. In both mushroom classes, after the extraction of water soluble polysaccharides, the residue obtained is richer in glucans (72 - 88 mol%), probably due to the presence of β-glucans, which have been described to affect viscosity in intestinal lumen affecting cholesterol accessibility.

Considering the importance of soluble fiber as food hypocholesterolemic ingredients, future work will need to establish if a deproteinization, which may occur during digestion, may influence hypocholesterolemic effects of water soluble fraction, containing proteins and soluble fibers, towards a decrease on cholesterol accessibility. Their regular intake, proteins and soluble fibers as mushroom based ingredient, may enable a management of cholesterol levels in humans, delaying the development of cardiovascular diseases.

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Dare 2 Change

Sustainability and Circular Economy

[S&EC 2]

'DELICIOUS LEFTOVERS: Upcycling brewing industry's yeast biomass for food flavour enhancement'

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Climate change and resource overuse require a transition to a cleaner and more efficient circular economy. At White Labs, we continuously focus on the optimization of our production processes towards a “zero waste” operation, namely reducing water utilisation and upcycling side-streams into new and higher added-value products. As a liquid culture supplier for the beverage industry, yeast products are our main side-stream, mainly expired yeast inventory. Yeast cells have been shown as a source of vitamins[1], umami-rich aminoacids[2], and the flavour active kokumi peptides[3].

Our work shows that these high density, single strain cultures can be converted into different forms of flavour enhancers by applying different treatments. In particular, long heat treatment at different temperatures produces umami-rich yeast extracts through autolysis. However, the yeast species and strains, but also growth conditions, play a significant role in the flavour profile, ranging from rich meat broth to refined soy sauce. The application of short heat treatments released kokumi-active peptides that improve flavour richness. Application of such upcycled products could significantly improve the flavour intensity and overall product acceptance in the alternative protein segment.

Overall, the current global challenges require that the brewing industry and all its partners find more energy-efficient value chains, and the valorization of the industry side-streams and by-products into new high value products can significantly contribute to the industry's financial and environmental sustainability.

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How much Bread waste can a Beer get?

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Annual bread production exceeds 100M tones, reflecting an estimated waste/loss of 10%. Bread waste/loss is alarming at different levels. First, it is an obvious waste of essential nutrients, considering a world problem of deficiency on cereals, aggravated by the war in Europe. Secondly, it is possible to count a whole range of losses regarding human and energetic resources. All efforts applied in the cultivation, harvesting, processing, packaging, and transport of both ingredients and final products must be taken in consideration, as we increase the carbon footprint of our industry. Some companies keep an active and continuous search to increase sustainable production of beer with staling bread incorporation. This replacement stands in 25% of the necessary malt to produce beer.

Since bread is rich in starch, there should be some viability in recirculating[1] its components for the production of fermented beverages. Bread starch is partially protected from the major enzymatic activity by the gluten network. Also, starch gelatinization creates different resistance to hydrolysis resulting in higher temperatures[2] for saccharification. Finding ideal hydrolysis conditions is the key to a higher yield of fermentable saccharides, meaning that an optimized hydrolysis grants an increased access to starch depolymerization to produce ethanol[3].

The final goal was defined in a successful 50% incorporation to brew a Pale Ale beer. To acquire a higher percentage of fermentable sugars from bread, adjusting times and temperatures on each phase of the process, with important enzymatic activity, is essential. To define the best conditions we have been evaluating ºBrix evolution of the wort in each phase.

Standing at the edge of a serious cereal crisis, consequently conducting to an aggravation of world hunger, a relentless search to new alternatives must be the agenda. Sustainable production supported by circular economy may just be the fastest and vivid solution to this threat.

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[S&EC 4]

Antioxidant and antimicrobial properties of PLA-based active packaging with pomegranate peels and extract

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Active food packaging' primary goal is to extend foodstuffs' shelf life, through a dynamic and continuous interaction between the package and the packaged food. In an emission active packaging, the objective is the gradual release of antioxidant and/or antimicrobial compounds into the food surface, to delay the natural foods' degradation. The active compounds can be extracted from several sources, such as aromatic plants, seaweeds, fruits by-products, among others. Since 50% of pomegranate is composed by peels, and since it is mainly consumed in juice and jam form, pomegranate peels may represent a considerable asset for the extraction of such compounds.

The principal objective of this work was to evaluate the antioxidant and antimicrobial properties of polylactic-based active packaging incorporated with 3% (w/w) of pomegranate peels (PLA/3PP) and 3% (w/w) of pomegranate peels extract (PLA/3PPE). For the in vitro antioxidant activity evaluation, 9.08 cm^2 of films were immersed in the food simulator, ethanol 95% (v/v), at 40 °C for 10 days. Then, the DPPH radical scavenging assay[1] was performed, as well as the total content of phenolic compounds[2], total content in flavonoids[3], content in punicalagin (A+B) and ellagic acid were determined[4]. Also, to fully determine the total content in punicalagin (A+B) and ellagic acid, the films were kept in methanol at 25 and 40 °C for 24 h. The antimicrobial activity of the films was evaluated with *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*, in accordance with ISO 22196:2011[5].

Results showed that PLA/3PPE presented a higher antioxidant potential and higher content in phenolic compounds and flavonoids. Only ellagic acid was detected in the active PLA-based films. Regarding the antimicrobial activity, both films presented antimicrobial activity against *S. aureus*.

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[S&EC 5]

Effect of pomegranate peels and extract in barrier, optical and mechanical properties of polylactic acid-based active packaging

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Being more than 50% of pomegranate (*Punica granatum L*.) constituted by non-edible parts, namely peels (50%) and seeds (10%), pomegranate is an excellent source of by-products. Its peels and seedspresent excellent antioxidant and antimicrobial activities and a high content of phenolic compounds, namely ellagitannins.

This work aimed to evaluate the mechanical and optical properties of two polylactic acid (PLA)-based active packaging with 3 wt.% pomegranate peels (3PP) or 3 wt.% pomegranate peel extract (3PPE). Allthe samples were produced on a laboratory scale with techniques and processing conditions used in industry. The production of packaging with flexible films is mainly carried out by tubular film extrusion. With this processing method it is possible to produce samples with molecular orientation and reduced thickness equal to that of the packages currently on the market. The structural and morphological characterization of the films were evaluated by FTIR and SEM, and the color by UV-vis. Water vapor transmission and mechanical properties were also measured. The color was measured by Shimadzu UV2401PC reflectance spectrophotometer. Water vapor transmission, oxygen permeability and mechanical properties were also measured.

The FTIR and SEM results indicate the incorporation of the pomegranate peels and peels extract in thePLA matrix, where PLA/3PPE showed better particle homogenization than the PLA/3PP. Regarding thecolor variations, the PLA/3PPE presented higher variations in terms of L*, a*, and b*. The incorporationof pomegranate derivates has a negative effect on the tensile strength and Young modulus, but a significant increase of the elongation at break for PLA/3PPE. The PLA film's water vapor barrier properties do not suffer any alteration with the incorporation of pomegranate extract.

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[S&EC 6]

From sulphite liquor production to valorization: challenges in vanillin crystallization

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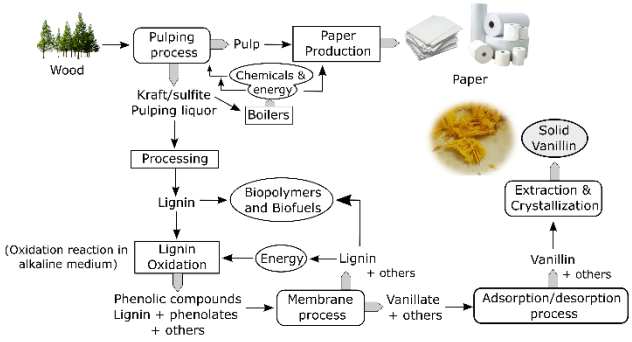
Vanillin is a potential food and pharmaceutical phenolic compound that can be produced from lignin derived from the wood pulping processes. An integrated process to produce added-value products by directing a stream of mother liquor from biorefineries has been developed in LSRE/FEUP [1, 2]. In this process, lignin depolymerization is attained by oxidation in alkaline medium using oxygen. The oxidized stream is submitted to ultra and nanofiltration to separate high molecular weight fractions from lower molecular weight species. The permeate from nanofiltration proceeds to adsorption/desorption experiments and a fraction rich in vanillin is obtained. This work studied crystallization process applied to an aqueous solution rich in vanillin obtained from the oxidation of isolated lignin from softwood sulphite liquor [3]. The crystallization yield and the vanillin crystals purity were determined, and the thermal stability of polymorphic forms was analysed.

The polymorphic form of vanillin was found to be dependent on the crystallization method as well as the temperature variation during the process. The slow evaporation method and cooling crystallization give origin to the stable polymorph Form I, with higher yields than swift cooling and evaporative process with heat that favour the nucleation of vanillin polymorphic Form II, with yields around 55%. Moreover, from evaporative processes, the vanillin crystals present a purity of about 95%, while for cooling processes the purity is slightly higher, with values around 99%. The melting points, observed in the DSC thermograms, of Form I and Form II of vanillin were found to be 82.6 ºC and 81.0 ºC, respectively. Proving that metastable Form II is less stable and highly energetic than stable Form I.

The evaporative and cooling crystallization processes studied proved to be consistent and valuable considering the obtained polymorphic forms of vanillin that present distinct physicochemical properties and consequently different industrial applications.



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Valorization of *Actinidia arguta* leaves as cosmetic ingredient: *In-vitro* and *in-vivo* safety and efficacy evaluation

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Actinidia arguta (kiwiberry) is a healthy fruit in exponential production. During kiwiberry production, leaves are removed to promote the fruit maturation, representing a by-product produced in huge amounts [1]. Our previously work reported the richness of kiwiberry leaves extracted by a green and eco-friendly technique – Microwave-assisted extraction (MAE) – particularly in phenolic compounds withantioxidant potential [2]. In addition, the demand for new cosmetic active ingredients based onsustainable principles arise as a trend. The aim of this study was to screen the in-vitro safety of the kiwiberry MAE extract on skin cell lines and 3D validated skin and ocular models (namely, EpiSkin™ and SkinEthic™ Human Corneal Epithelial Models, respectively). Briefly, the kiwiberry leaves were extracted according to Silva et al. [2]. Cell viability assays were performed in keratinocytes (HaCaT) andfibroblasts (HFF-1) using different extract concentrations (0.1 – 1000 µg/mL). The MTT results revealedthat the hydroalcoholic extract did not decrease the HaCaT viability, while the HFF-1 viability was above90%. Considering these results, the extract was tested on 3D skin and ocular models. The viability achieved in both models was, respectively, 55.18% and 101.15%. Regarding the IL-1α released after exposure to the extract, it was 0 pg/mL for the skin model, while for the ocular model was 35.60 pg/mL. These values were significant different from the positive control employed that was sodium lauryl sulfate(522.90 and 55.19 pg/mL, respectively), being the extract classified as non-irritant for both models. Afterwards, a patch test assay performed in human volunteers (n=10) guaranteed the absence of allergic or irritant effects.
These results demonstrated that the kiwiberry leaves MAE extract is not irritant to skin and eyes, provingit potentialities as cosmetic ingredient. Further studies, such as anti-wrinkles activity, should beperformed to ensure the skin effects on final consumers.

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Integrated strategy for the valorisation of agri-food waste

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The fruit and wine industries represent important sectors in Portugal, generating different products and allowing numerous job opportunities. However, at the same time, they also generate a considerable amount of bioresidues as peels, skins, peduncles, and non-compliant fruits that are not properly recovered by the industry (1). The accumulation of this waste biomass often causes potential environmental impact, along with the loss of material that can be processed to produce a high number of value-added products, such as foodstuffs, biofuels, chemicals, pharmaceuticals, and other molecules of interest (2). The main objective of this project is to develop innovative strategies for the valorisation of by-products from this sector, optimizing the processes of extraction of chestnut starch, dyes and phenolic compounds, hydrolysis of lignocellulosic materials, fermentation, and alcoholic distillation, so that their application in the industrial environment can be simple, easy to perform, and low cost. The project is composed of three entities of the national scientific and technological system – University of Trás-os-Montes and Alto Douro (UTAD), University of Minho (UM), and Polytechnic Institute of Bragança (IPB) – and two companies – AGROAGUIAR, a company that grows, acquires, processes, and distributes Portuguese fruits and nuts for the national and international markets and DESTILDOURO, a company specialized in the production and commercialization of brandies and alcohol. In this context, the specific objectives of the project are: to produce alcohol for pharmaceutical use from noncompliant fruits (strawberry, raspberry, blueberry, and blackberry) and by-products (peel, fruit skin, leaves, and fruit stalks); to extract value-added compounds (dyes and phenolic compound-rich extracts) and to optimize processes for industrial application. In addition, the project is related to the main Sustainable Development Goals of the 2030 Agenda, from ONU, namely in the reduction of waste generation through reuse.

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[S&EC 10]

Extending poultry meat shelf life through the application of *Cynara cardunculus L*. leaf extracts

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Cynara cardunculus L. (Asteraceae), commonly named cardoon, is a multipurpose crop that includes three varieties, the globe artichoke (var. *scolymus* (L.) Fiori), the cultivated cardoon (var. *altilis* DC.), and the wild cardoon (var. *sylvestris* (Lamk) Fiori). Its flower is normally used as vegetal rennet in the production of some cheeses and its leaves, the main by-product generated, are known for its excellent antioxidant and antimicrobial activities[1]. These properties may be an asset in the food industry as cardoons' leaves may be used to delay lipid oxidation and microbial growth, thus prolonging foods' shelf life.
Therefore, this study aims to evaluate the effectiveness of cultivated cardoon leaves and the globe artichoke leaves ethanolic extracts, on poultry meat preservation. Poultry meat was mixed with the different extracts at a concentration of 1% (w/w) and stored under refrigeration (5 °C ± 2 °C) for 15 days. The microbiological growth was evaluated through the assessment of the total mesophilic aerobic microorganisms, total psychrotrophic aerobic microorganisms, and Enterobacteriaceae. The physicochemical characterization was evaluated through moisture, pH, acidity, colour and Total Volatile Basic Nitrogen (TVBN), and the lipid oxidation by Thiobarbituric Acid Reactive Substances (TBARS).
Both extracts were effective in retarding microbial growth by maintaining constant pH and level of acidity. After 15 days, poultry meat with both extracts showed a difference up to 11 log CFU/g to control samples (without extract). Also, both extracts were able to reduce the lipid oxidation of the poultry meat when compared to the control samples, at the end of the assay. The colour of extracts can be a limitation due to the greenish-yellow colour that is seen in the meat, although it was more evident in the sample with the cardoon extract. Overall, cardoon extract was the most effective in extending poultry meat shelf life.

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Coffee pulp produced in Azores - a source of bioactive compounds

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During coffee processing, several byproducts are discarded [1], despite the presence of compounds of interest, including carbohydrates: free sugars, oligosaccharides, and polysaccharides. Oligosaccharides can contribute to a prebiotic effect [2], potentially contributing to a risk of metabolic syndrome reduction [3]. This work aimed at the characterization and extraction of carbohydrates from a coffee pulp produced in Azores. Defining the characteristics of all fractions can define applications and provide additional sources of income for farmers. But, to maintain the food grade, the byproduct needs to be stabilized. In this work, two methods were used: freeze-drying and 40 °C oven-drying.

Mature coffee cherries were manually collected from *Coffea arabica* plants ("Caturra Vermelho" cultivar) produced in S. Miguel, Azores. The fruits were washed with tap water, dried with blotting paper, and manually depulped. The pulp was then divided in two groups, freeze-died and oven-dried at 40 °C. The samples were treated in a sequential procedure: 1) ethanol (85%, v/v) at 80 °C for 10 min, 2) hot water at 80 °C for 2 h. All extracts were characterized for their neutral and uronic acids carbohydrate composition. A colorimetric analysis (dinitrosalicylic acid, DNS) allowed to quantify the reducing sugars.

The reducing sugars accounted for 29.6±0.4% (freeze-dried), and 26.4±1.6% (oven-dried). The total sugar content showed 43.9+3.6% (freeze-dried) and 29.9+5.8% (oven-dried), respectively. Most of the it, corresponding to free sugars (glucose, fructose, and arabinose) that were recovered in the ethanolic extract. The hot water supernatants showed a high content of uronic acids (50-70%), consistent with a pectin-rich extract. These results showed stable samples, with distinct composition upon the extraction solvents, allowing to enrich extracts with different carbohydrates. From this work, it can be expected Azores pulp to be a sustainable source of pectin, but also oligosaccharides and free arabinose, a trait that can be explored to improve conditions in the gut.

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Exploring *Cynara cardunculus* L. potential for the food industry: the antioxidant pattern

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The perennial plant *Cynara cardunculus L.* (cardoon), which comprises three botanical varieties like wildcardoon (var. *sylvestris* (Lamk) Fiori), cultivated cardoon (var. altilis DC.), and globe artichoke (var. scolymus (L.) Fiori), is a multipurpose crop, native to the Mediterranean area[1]. Cardoon is mostly knownfor its flower that is used as milk clotting in soft cheese making, where the leaves are the main by- products. The leaves, rich in bioactive compounds, are used in traditional medicine and have interesting antioxidant and antimicrobial activities[1]. Therefore, cardoon leaves have potential interest in the food industry to extend foods' shelf life by delaying lipid oxidation and microbial growth.

This study aims to determine the antioxidant activity and the total content of phenolic compounds and flavonoids of cultivated cardoon and globe artichoke leaves extracts, both methanolic and ethanolic. Therefore, two different assays were performed: the DPPH free radical scavenging and the β-carotenebleaching assays for the evaluation of the antioxidant activity. Also, the Total Phenolic Compounds (TPC) and the Total Flavonoids Content (TFC) were determined.

Cardoon extracts, both ethanolic (2.1 mg/mL) and methanolic (0.8 mg/mL), presented lower EC50 thanartichoke extracts ($EC50_{EtOH}$= 3.9 mg/mL; $EC50_{MeOH}$= 1.6 mg/mL), which means greater antioxidant capacity. For the β-carotene assay, cardoon extract (AAC_{EtOH}:448.06; AAC_{MeOH}:279.67) presented a higher antioxidant capacity coefficient (AAC) than the artichoke extract (AAC_{EtOH}:90.98; AAC_{MeOH}:114.97). Accordingly, cardoon extracts (EtOH: 81.98 mg GAE/g; MeOH: 112.84 mg GAE/g) also had a higher content of TPC than artichoke (EtOH: 49.14 mg GAE/g; MeOH: 29.79 mg GAE/g). The same can also be observed for TFC, where cardoon (EtOH: 145.47 mg ECE/g; MeOH: 129.27 mgECE/g) presented greater flavonoid content than artichoke (EtOH: 81.33 mg ECE/g; MeOH: 21.24 mg ECE/g). These results confirms that cardoon leaves are a natural source of antioxidant compounds thatcan be exploited by the food industry.

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Vegetable lactic acid fermentation: a value chain approach to fightfood waste and increase healthy food offerings in retail

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Fighting food waste is an ethical obligation of both consumers and companies. Consumers want products that promote circularity and the upcycling of agricultural products that otherwise would be used as compost [1]. In the project **FermVegAlgae**, an innovative collaboration between Mendes Gonçalves, MC Sonae and ISA led to the development of lactic acid fermented vegetable mixtures with the intent to reach commercial status. A connection was established with agricultural operators to use excess vegetables to generate lactic acid fermented products. Further inquiries exposed white cabbage, red cabbage, pepper and carrot as vegetables with excess production.

A fermentation strategy was established at laboratorial scale to minimize fermentation time and contamination, while maximizing organic acid production: a consortium of four lactic acid bacteria strains was employed [2]. Several trials were performed with different variables: inoculum vs natural microbiota, salt concentration and vegetable mixture. The success of the fermentation was established as the stabilization of pH and acidity. Although good fermentation results were achieved at 0,3% (w/w) NaCl concentration with inoculation, the best scenario was achieved at 1% (m/m) salt concentration with inoculation, which allowed increased contamination resistance and therefore better scalability. To maximize product shelf-life, a pasteurization strategy was established with previous addition heat resistant probiotic spores [3]. Four different vegetable based prototypes were prepared, including a sauce and a spread. Preliminary consumer studies were performed and will be used to maximize commercial approval, namely in acid perception and flavour acceptance (such as garlic flavour). Preliminary commercial technical sheets and labelling studies were already performed and include “source of fiber” claims.

In MG facilities, the industrialization of the production process is underway to obtain high reproducibility. This project creates several products with high market potential that not only increase the sustainability of the food production chain but also creates a tasty, affordable and healthy offering for consumers [4].

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[S&EC 14]

Integrative and sustainable strategy for the valorization of pumpkin by-products

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The food industry has sought to meet consumers' demand for practical, ready-to-eatfoods capable of meeting daily nutritional needs and providing pleasure and satiety. However, the industry still relies on artificial additives to preserve and improve food properties, due to the lack of effective natural analogues, which face stability and availability limitations [1]. Aiming at the objectives of the circular economy and the promotion of more sustainable agro-industrial processes, the ambition of this project is to develop a new pumpkin fruit pulp formulation functionalised with a biobased preservative extracted from pumpkin by-products. For that purpose, production protocols will be established to ensure high agronomic performance and high-quality pumpkins. Meanwhile, pumpkin pulps will be evaluated in terms of nutritional value and the by-products, as seeds, peels, and fibers, will be assessed for their content in preservative compounds. The recovered, refined, and stabilized preservative compounds will further be incorporated in the pumpkin product formulated with the pumpkin elite cultivars. In addition, the quality assessment of pumpkin fruit pulp during shelf-life and the waste and wastewater management are targeted. The proposed project involves multidisciplinary research groups (IPB/PT, MORE/PT, UTH/GR, GFV/GR, CBBC/TN, ATB/DE, CRAPC/DZ, BU/EG) with various expertise in the food and farming sector, and the company DECORGEL/PT, which will further process the pumpkin pulp to be "market ready", within the consortium PRIMA Section 2 - Multi-topic 2019: PulpIng (PRIMA/0007/2019). The described aims are in line with the EU Biodiversity Strategy of 2020 and 2030 Agenda for Sustainable Development, and intend to address food safety/security issues and promote circular food systems in Mediterranean regions.

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[S&EC 15]

Perceptions and attitudes of Portuguese consumers towards local and seasonal foods

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The consumer is the endpoint of any supply chain, and he has become the focus of concern considering the food sustainability challenge the world is facing [1,2]. The consumption of seasonal foods to reduce long-distance imports and the unseasonal local production to reduce the environmental footprint of consumer food choices remain relatively little discussed and investigated [3]. Although there is not a clear legal definition for local and seasonal products, these terms are increasingly used at the retail level to support fresh fruit and vegetable sales [4]. To promote local and seasonal food, it is crucial to explore how consumers understand the concept of local food. This includes the benefits and/or disadvantages they perceive and the values they associate with.

This work aimed at investigating consumers' attitudes, perceptions, and beliefs about seasonal and local food. Being crucial to understand how consumers think about the terms "local" or "seasonal" in relation to food.

The Lime Survey platform was used to create and implement a questionnaire. The questionnaire was divided into different sections, and it was applied in the Metropolitan Areas of Lisbon and Porto, following a convenience sample of over 400 Portuguese participants.

Results show that the conceptualization of “local” is divided into three dimensions: Geographic, Origin and Traditional. The Traditional dimension, including local markets and producers, was significantly more associated with the concept. For ‘seasonal’, the simpler description of a ‘product available in specific periods of the year’, was the most strongly associated with the concept, while being recognized by over 80 % of the participants.

This information can be used to develop marketing strategies, education, and nutrition programs that will help to improve the development of food systems that are more localized and/or community-based.

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[S&EC 16]

Valorization of spent-grain, oat and strawberry puree co-products as ingredients in cereal bars

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Worldwide food industry is facing resources scarcity and raw materials prices' increase. Meanwhile, from food manufacturing processes can result in food waste, which imply resource losses, economic, social and environmental negative impacts. A possible action to minimize this problem is to try to re-incorporate the co-products in new food products to pursue a "zero waste economy". Besides that, veganism and ethical issues to alleviate ecological problems, and health concerns are trending among consumers.
The objective of this work was the evaluation of three co-products recovered during manufacturing of oat drink (oat flour), beer (spent grain) and sieved strawberry puree (seeds) in the formulation of a nutritious vegan cereal bar. The amount of each ingredient was adjusted to obtain the best possible Nutri-Score while maintaining a pleasant taste and texture. Eggs were replaced by hydrated flax seeds to preserve binding properties. Wheat flour (14%) was used in Control (CT) and in 5% strawberry seeds (SS) formulations. Wheat flour was replaced by 14% oat flour (OF), or 12.6% oat flour and 1.4% spent grain (OS) in two other formulations. CT, SS, OF and OS formulations were evaluated by a consumer panel of 31 people using hedonic scales. Texture properties (hardness and fracturability), color, and water activity were also determined after production and during storage. Adding strawberry seeds increased pink color, red fruits flavor, and crunchiness, resulting in an increased appreciation and Nutri-Score A, while spent grain and oat flour deepened brown color. Hardness of OF and OS bars were lower than CT and SS bars. Processing conditions need to be addressed since inadequate cooking will affect negatively the organoleptic profile of bars. Nevertheless, consumer panel appreciated all the versions, meaning that it is possible to completely replace wheat flour for by-products flours without depreciation in the taste of the bar.

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[S&EC 17]

Upcycling of spent grain and oat by-products as an ingredient in cracker's formulation

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Nowadays, increasing attention is given to the valorization of agro-industrial by-products and their transformation into valuable new products, to reach the ultimate model of a "zero waste economy". Consumers' awareness of healthy diets is rising and food industries are focusing on the formulation and development of functional products, in combination with cost reduction through waste upcycling.
This study aimed to verify the feasibility of spent grain and oat by-products' valorization as raw materials in formulating crackers, with acceptable physical and sensory characteristics. Several formulations were tested with different concentrations of spent grain flour (5-10% w/w), oat flour (5-10% w/w), salt (0.2-1% w/w) and partial replacement of oil with pre-hydrated linseed. Nutri-Score was calculated to help in the design of crackers with better nutritional value, since the initial formulation had 7.5% oil and a Nutri-Score C. Three formulations with Nutri-Score B and A were selected to evaluate their organoleptic properties, using a nine-point hedonic scale and ranking test, with a consumer panel of 21 people. Texture properties (hardness and fracturability), color, and water activity were also determined after production and on 7th day.
It was concluded that crackers´ formulation with higher amount of spent grain was less brittle and had a darker color than the remaining formulations. Hardness and water activity (0.439-0.470) was very similar amongst formulations. In general, parameters such as flavor, salt intensity, crunchiness and oil intensity had good acceptability, particularly crunchiness. Crackers made with 7.5% spent grain flour, plus oat flour, and with 1% salt had the highest rate for overall acceptability (6.8), although with poor Nutri-Score (B) which can be improved by lowering salt content to 0.7%. The development of crackers with the incorporation of spent grain and oat flours from by-products, is a feasible strategy for upcycling these ingredients, creating more nutritious and sustainable food products.

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Urban hydroponics for sustainable locally-produced vegetables

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Modern agriculture faces the great challenge of responding sustainably to the growing world demand for food. An increase of the human population is expected in the next 40 years [1, 2], being estimated at 9.1 billion people by 2050 [1], reaching 10 billion within the first few months of 2056.

Hydroponic systems will have, in a near future, an important role on quenching the increase in food demand, as they have the potential to mitigate local demand for vegetables. Locally producing vegetables will allow: i. a more efficient geographical response to local food demand by; ii. deployment of fast-implementing food systems with better phenological phase times and; iii. to free soil resources for other non-hydroponic-suitable crops.

Hydroponics are a milestone of the precision agriculture era, but, nevertheless, still require technological development to respond to the required level, adequately. Nutrient uptake is one of the most important variables to control; nevertheless, current available technology falls short of measuring Nitrogen, Phosphorus and Potassium (NPK) reliably, namely regarding what it concerns to the chemical network interferences, which are, often, disregarded or wrongly assessed.

The development of reliable NPK sensors for fertilizer uptake assessment that consider chemical network interferences [3, 4] – inherent to the co-existence of different fertilizers – will allow to achieve maximum crop yield and turnover rate, as well as establish an improved fertilization plan (quantity and composition) adjusted to the crop's phenological state. These devices will also allow the spawn of micro- green hydroponics, with local influence and coverage area. Urban food-systems can be installed on, e.g., building tops, acting as small-scale continuous green vegetable production flow systems. The concept of Farm2Fork [5] will achieve its apex, delivering a neglectable chain of transport times while maximizing the delivery of nutritional value to the consumer. Retail sellers will be able to claim vegetable- freshness as an advantage, as they will be within an arm's reach for the consumer.

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[S&EC 19]

Project “Innovative down-scaled food processing in a box (FOX)” main results on sustainable processing and packaging of fresh-cut fruits and vegetables combinations

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The Project “Innovative down-scaled Food processing in a box (FOX)[1]” coordinated by the German Institute of Food Technologies (DIL), brings together innovative soft processing technologies so that growers can use their fruit ans vegetables surpluses to develop products with greater added value. Its goal is to create shorter, more sustinable aupply chains by adapting large-scale processing technologies to small, flexible mobile container units, thereby helping to cut food waste and reduce the need to transport produce to centralised processing facilities.
Four types of unit are being developed based different technologies and oriented to different types of products and regions. All the prototypes will be available by 2023, when demostrations will take place in different regions across Europe, denoted Food Circles. The Food Circles link a specific FOX processing technology with a regional (short) food production in order to demostrate the technical, economic and social viability of the FOX approach. These are located in Spain, Czech Republic, Poland, France, Germany and the Netherlands. One of the factors that makes FOX unique is that the Project follows a general approach called the “multi-actor approach”, in which end users, such as farmers and consumers, are fully involved during the whole project.
The results are focused on the mobile container unit developed for conditioning and packaging different combinations of fresh fruits and vegetables making use of sustainable minimally processing technologies, and new sustainable packaging systems (primary and secondary). Depending on the kind of packaging system and its function, different ecodesign strategies were considered.

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[S&EC 20]

Towards more sustainable and circular food production systems: Life cycle assessment of pumpkin pulp production using conventional and alternative preservatives

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Food production systems contribute to several environmental impacts, such as climate change, land use, depletion of freshwater resources, among others [1]. These impacts can originate from the entire supply chain. Hence, the whole life cycle of crops must be considered and life cycle assessment is a suitable tool for this purpose [2].
In this domain, agricultural waste resulting from food production is a major concern, as not only resources are irrationally expended when the whole product and by-products are not used, but also, inappropriate treatment of crop and food wastes can result in further environmental impacts, deeming their valorisation fundamental [3]. This study is integrated in project PulpIng – Development of pumpkin pulp formulation using a sustainable integrated strategy – where the main goal is to develop a new preservative, based on pumpkin crop by-products, and substitute typical preservatives, some of which are obtained from non-renewable petroleum resources, while simultaneously closing the cycle and avoiding the generation of this type of waste. Bio-based preservatives have previously shown to be interesting alternatives to synthetic ones, resulting in a reduced carbon footprint [4].
Hence, this study assessed the potential environmental impacts resulting from the industrial production of pumpkin pulp. For this purpose, the following stages of the life cycle were considered: cultivation and pulp production (cradle-to-gate). First, the traditional technique for cultivation, in which pumpkin by-products are considered as waste, and the production of packaged pumpkin pulp, using traditional preservatives, were assessed. Then, the same cradle-to-grave assessment was performed considering that pumpkin by-products were used to produce a preservative that will substitute the traditional alternatives in the pumpkin pulp.
Thus far, preliminary results for the cultivation phase have been obtained. This work is expected to contribute to the environmental sustainability of pumpkin pulp production, and results might be useful for other food production systems.

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[S&EC 21]

Integrated strategy for promoting the socio-economic sustainabilityof the cultivation and consumption of Montesinho mushrooms

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Montesinho Natural Park represents a mountainous area with a unique mycological heritage. Among the approximately two hundred edible mushrooms found in this protected area, most have been consumed since antiquity, as in addition to their exquisite flavor, and are highly appreciated for their nutritional value [1]. These represent interesting sources of protein, dietary fiber, and minerals, in addition to containing high added value compounds for the food/nutraceutical/pharmaceutical industry [1, 2, 3, 4]. However, as wild species, mushrooms have a seasonal frequency, which limits the availability of this product in the mountains, a problem made worse by climate change. On the other hand, unsustainable mushroom harvesting practices and illegal trade in high added-value species have also been common practices with negative impacts on the ecosystem and on the regional and national economy.

Many restaurants have decided not to include them on their menus to protect their business and the health of their consumers due to all these issues and the inability of local pickers to guarantee the authenticity/safety of the mushrooms they pick. Thus, it is suggested that edible mushrooms be produced ex situ in a controlled environment, resorting to the recycling of forest cleaning material to produce substrates. To guarantee the excellent quality of the species produced and the preservation of their original characteristics, a detailed nutritional, chemical, and bioactive characterization is being carried out. Increasing consumer trust and loyalty in products is the objective of creating the “Safe2Taste” quality and safety seal, which guarantees traceability of the entire production chain.

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Spirulina (*Arthrospira platensis*) protein fractions as sustainablefood ingredients

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Proteins are widely used as stabilisers, emulsifiers, foaming, and gelling agents in food formulations. The most used proteins in the food industry are from animal sources. However, there is an increasing demand for sustainable food ingredients to face the growing consumer awareness of environmental andhealth issues. Besides, more people are changing their diets to vegan, vegetarian or flexitarian. For these reasons, cell-based proteins (e.g., microalgae) are great alternatives to animal-based counterparts. Microalgae are considered sustainable sources of proteins since they do not need arableland to be cultivated and do not compete with food chains. They can capture CO_2 from the atmosphereand produce protein at higher yields per unit area than traditional crops. Among microalgae, Spirulina (Arthrospira platensis) has a large content in proteins (50-70%), vitamins, minerals, and lipids that can be extracted under a biorefinery approach [1,2]. Thus, this work aimed at extracting protein fractions from Spirulina under an integrative perspective to be used as food ingredients.

After lipids fraction extraction, the protein-rich extract (SpE) was obtained from the Spirulina defatted biomass by applying ultrasound and stirring, followed by isoelectric precipitation. SpE (4 wt%) formed and stabilised oil-in-water emulsions (O/W) at a 30/70 weight ratio. The emulsions remained stable against creaming for up to 30 days of storage, showing SpE's potential to replace animal-based emulsifiers in food emulsions [1]. The extracted lipids fraction can be destined for biodiesel production or essential fatty acids production, adding greater value to the biomass.

Another protein fraction, rich in a natural blue pigment (C-phycocyanin, C-PC), was obtained with food-grade purity by Spirulina biomass immobilisation in calcium-alginate beads. C-PC can be applied as a natural colourant or stabiliser in food formulations, and the leftover beads can be used as feed or food supplements, valorising the complete biomass and generated co-products.

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[S&EC 24]

Disclosing the potential of hydrosols as sustainable alternatives

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New trends focusing on sustainability drive industries to follow a green pathway by reducing their wasteand use natural products. In this scenario, hydrosols, the secondary product of the essential oil industry, emerge as a natural source presenting promising bioactive properties. Hydrosols are complex mixturescombining essential oil traces with other hydrophilic bioactive components, which due to their unique properties, like antimicrobial, can find several sustainable uses in industries such as food, pharmaceutical, and cosmetics. Therefore, this work aims to investigate the Melissa officinalis Linnaeushydrosol enabling its valorisation in line with the concepts of the circular economy. Thus, the work wasdivided into two lines of study: a) chemical and biological characterisation for hydrosol application as anantimicrobial agent; b) study of the potential to be applied as a cheaper substrate to produce the microbial polysaccharide xanthan gum using the bacteria Xanthomonas campestris.
Regarding the chemical composition, the citral isomers were identified as the major components relatedto the antimicrobial capacity. This capacity was corroborated by the antimicrobial assays that showed asignificant reduction in microbial growth against Escherichia coli, Staphylococcus aureus, and Candida albicans. Regarding the biopolymer production in the presence of the hydrosols (10% v/v), it was possible to achieve an increase in the production when compared to the control (substrate without hydrosol), which may be related with the bioactive compounds acting as a stress factors leading to microorganism's response mechanism. In brief, these results indicate that hydrosols are good candidates to be applied in encapsulation/hydrogel systems using natural polymers (e.g., xanthan gum produced by recycling a waste), closing a green production cycle; or to be included directly in different products (e.g., for food preservation as natural antimicrobials).

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[S&EC 25]

Identification of molecular rapid methods for hidden insect infestation determination in rice

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Rice is one of the most consumed cereals worldwide and insect infestation is one of the main reasons for the quantity and quality loss during rice storage [1]. Some species of adult insects are especially adapted to attack whole rice grains and normally spend a considerable part of the life cycle, including the entire larval feeding period, inside grains. Other species in their larval stage create an entrance hole within a grain, covering it after entry and an adult exit hole is created after the process of pupation, from which it then emerges. These insects constitute the hidden insect infestation which visual inspection cannot successfully detect, so, the identification of rapid detection and monitoring methods is of greater relevance for all the rice value chain stakeholder´s.

There are several methods to detect the presence of hidden infestation in rice. The most conventional techniques (ISO 6639:4) [2] include determining carbon dioxide production, the ninhydrin method, the whole-grain flotation, X-Ray and the acoustic method, but these are time consuming and obsolete techniques. New molecular techniques such as DNA bar-coding, species-specific polymerase chain reaction (PCR) and real time PCR or quantitative PCR have potential application in industrial scale.

In this work we will focus on the development of rapid molecular techniques to detect relevant insect species that are commonly found in rice, such as *Sitophilus oryzae* and *Sitophilus zeamais*, so that in the future it can be validated and eventually implemented in industry laboratories.

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[S&EC 26]

Elaboration of films from plant residues: a more sustainable alternative to the environment

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Traditional food packaging is usually made of petroleum-based plastics and this type of packaging is nonrenewable and nonbiodegradable [1]. Thus, the increase in research involving eco-friendly packaging, such as the production of biodegradable films, has been increasing [2]. Plant agro-industrial wastes, such as peels, stalks and bagasse, have been studied as sources of bioactive compounds to act as antimicrobial, antioxidant, aromatic and/or indicative agents [3], and may also be used as sources of polymeric matrices to develop films.

Thus, the main objective of this work was to develop films using plant biomass residues, obtained from production of eucalyptus, licorice, thyme and sage extracts, as a polymers source. The plant residues were characterized and different films were produced. For this, residues (1%), alginate (1%) and glycerol [1% (v/v)] were used to develop films. Then, the antimicrobial activity of the films was tested against bacterial species *Escherichia coli* and *Staphylococcus aureus.*

The results showed that the residues consist, mostly of insoluble fibers ranging from approximately 55% to 79%, with lignin being the main constituent ranging from approximately 33 to 50%. Furthermore, it was possible to obtain films from plant residues, but the formulations need to be improved to assign better characteristics (physical and mechanical) to the films. The films did not show antimicrobial activity against the tested strains.

Nevertheless, the developed films can be considered potential solutions to be applied as biodegradable active packaging. However, more studies are needed to support this hypothesis, especially with the addition of natural antimicrobials. In addition, the use of these residues for film's production is a more sustainable alternative to the environment than conventional plastics and even adds value to plant agro-industrial wastes.

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Red grape pomace extract: bioactive potential against bacteria, fungi and SARS-CoV-2

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The outbreak of COVID-19 disease caused by SARS-CoV-2 forced the scientific world to search for new alternatives to help control the virus. Grape pomace, which is an industrial residue obtained from the winemaking process, has bioactive compounds derived from the grape [1], which can be a starting point for research on the use this residue as a source of new antiviral agents. Thus, the objective of this study was to obtain an extract from the by-product of red grapes discarded by the wine industry in Portugal to be used as an antiviral agent with possible application in disinfectants, fabrics or other materials.

The red grape pomace extract was obtained by maceration in a hydroethanolic solution (ethanol:H_2O 50:50 v/v) under optimized conditions and submitted to freeze drying. The extract was tested against pathogenic bacteria and a variety of fungi, and the antiviral activity was evaluated for SARS-CoV-2 in Vero cells.

The extract showed antimicrobial activity against a large number of bacteria tested, e.g., *B. cereus* (1.56 mg/mL), *L. monocytogenes* and *S. aureus* (3.125 mg/mL), and *E. coli* and *S. Typhimurium* (50 mg/mL). Regarding its antifungal potential, this extract did not show inhibition against the environmental fungi tested, however, it inhibited two of the tested dermatophytes. *T. mentagrophytes* and the yeast *M. furfur* at a concentration of 6.25 mg/mL and 50 mg/mL, respectively. Regarding the antiviral activity, the results achieved for the viral titer was 6000 PFU/mL, the antiviral activity for SARS-CoV-2 was 1.36 ± 0.15 Mv and the percentage of reduction was of 95.38 ± 1.54%.

The results showed that the obtained extract revealed consistent results of antiviral activity, presenting a potential for applications against SARS-CoV-2. In addition, it showed potential against some bacterial pathogens and fungal dermatophytes. Further studies are required for the validation and application of this extract.

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[S&EC 28]

Honey and related by-products as biomonitoring matrices of anthropogenic contaminants

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Honey has been a popular and functional food due to its antioxidant, antimicrobial, anti-inflammatory and anticancer bioactive components. Moreover, the recognized importance of beekeeping activities are in line with the Sustainable Development Goals 2, 12 and 15, aiming the promotion of sustainable agriculture, quality production, and sustainable use of terrestrial ecosystems [1]. However, emerging concerns around the possibility of contaminants occurrence in honey, arising from anthropogenic activities (urbanization and intensive agriculture practices) [2, 3], have to be considered. Two distinct approaches should be addressed, namely the problematic of having contaminated food products and the possibility of using honey, and by-products, as bioindicators of persistent human-hand contaminants in the ecosystem. Biomonitoring of abiotic stressors or anthropogenic contaminants in the environment using hive products are being reported proving that this possibility may be used for policy research. In addition to the occurrence of contaminants, including pharmaceutical residues, the use of bee products to assess the antimicrobial resistance genes dissemination is also an issue of growing interest [4].

The present work pretends to provide an intensive and complete review of the analytical strategies in place to monitor the presence of anthropogenic contaminants in honey, and related products, to define a starting point for new developments. Furthermore, it is consensual that the use of apiary products to evaluate environmental persistent contaminants can provide representative overview of ecosystem pollutants.

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Opuntia ficus-indica as potential source of pectin: optimizing the extraction yields

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Opuntia ficus indica (OFI) is a plant of the Cactaceae family that can grow in arid and semi-arid lands. Cladodes, the vegetative parts of the plant, have an interesting content in pectin. Pectin is a complex polysaccharide that is used as gelling and stabilizing agent in food industry. Pectin extraction is affected by: pH, time, temperature, solvent, solid/ liquid ratio [1,2]. Thus, the aim of this work was to optimize the pectin extraction from OFI cladodes.

For pectin extraction the procedure comprises the general steps: washing/cutting cladodes; mixture with solvents; centrifugation; precipitation in ethanol; drying. In this work the following parameters were tested: solvent (water, acetic, ascorbic and citric acid), pH (1.5–7), extraction temperatures (70-90 ºC), extraction time (40-60 min), and L/S (Liquid/solid ratio) (5-15). Extractions were performed in peel and pulp of cladodes.

Preliminary studies indicate that the pH does not affect the extraction yield. But more pectin was extracted from peel (average of 8.5% w/w db) than from pulp (average of 6.6% w/w db). Extraction with citric acid showed a higher pectin yield (average of 9.1% w/w db) than acetic acid (average of 6.5% w/w db) or water (acidified at different pH's, average of 7.5% w/w db). The extraction yield obtained using ascorbic acid demonstrated similarities with the use of citric acid as solvent. The remaining extraction parameters are still under study but first round of results point out for the use of an extraction time and temperature of 80 min and 50 ºC, and a proportion of L/S of 10. Yet, in this assessment only yields were taken into consideration. To identify an optimized procedure to extract pectin from OFI, the characteristics of the pectin should also be considered, which is also being studied so that pectin from OFI can emerge as alternative to fossil-based plastics to produce new packaging materials.

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Evaluation of the antimicrobial activity of pomegranate by product extract against bacteria

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Large quantities of food waste, namely peels, seeds and fruit pits, are produced daily in processing products of plant origin. Although they are discarded and neglected they are a valuable and biodegradable natural resource, with great economic potential. In their composition there are bioactive compounds that are known to regulate metabolic processes, through their antioxidant and anti- inflammatory activities, some of these compounds also have antimicrobial properties [1]. In this sense, one of the strategies addressed to avoid lack of food, reduce food waste taking advantage of the residues is to add value to byproducts, exploring their applications [2]. Thus, the objective of this work was to evaluate the antimicrobial activity of the aqueous extract of pomegranate peel on bacteria. For this, the disk diffusion method was used to evaluate the antimicrobial activity of the extract obtained from the pomegranate byproduct. Bacterial suspensions were prepared and adjusted to 0.5 McFarland standard scale [3]. Then, the suspension was spread in Petri dishes with Muller Hinton Agar (MHA) medium. Sterile filter paper discs (9 mm) with 20 µL of the extract were placed on the agar surface. Plates were incubated at 37 °C for 24 h, with the exception of Campylobacter jejuni that was incubated at 41.5 °C for 48 h under microaerophilic conditions. Gentamicin (10 µg/disk) and sterile ultra-pure water were used as positive and negative controls, respectively [4]. The results obtained with the antimicrobial susceptibility test by disk diffusion found that the aqueous extract of the pomegranate byproduct inhibited the growth of the bacteria Pseudomonas aeruginosa, Staphylococcus aureus and Campylobacter jejuni evidencing the great possibility of applying the extract in a product with natural antimicrobial potential.

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Folgasão variety: Evaluation of grape stems, grape pomace, andvine shoots extraction efficiency

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The wine production produces different type of by-products such as grape stems, grape pomace (skins and seeds), wine lees, vine shoots, among others [1]. Few studies have already proven that winery by- products can be a source of natural antioxidants, especially due to the fact that they possess phenolic compounds [2]. These residues can be reused as new ingredients in food, additive substitutes, and pharmaceutical industries. For that reason, scientific foundation for the comprehension of extraction methods efficiency is essential for starting the reuse of these by-products in a large scale [2]. Nowadays diverse extraction methods are studied using different solvents and extraction conditions [3]. Ethanol has been used as an extractive solvent due to its natural presence in wines, its safety behavior, and its low toxicity when compared with other organic solvents, such as methanol [4].

The aim of this study was to use eco-friendly solvents and compare the polyphenolic extraction efficiency, between five different solvents proportions, namely %Ethanol:%H2O, v/v: (100:0), (75:25), (50:50), (25:75), and (0:100). For that, the phenolic content (ortho-diphenols, total phenolic content, and flavonoids) and the antioxidant capacity (FRAP, DPPH, and ABTS) of extracts from stems, pomace, and vine shoots from Folgasão variety of the 2021 harvest from the Douro region were characterized, using spectrophotometric methods according to the methodologies already employed by our research group [5,6].

The best combination solvent that allowed to obtain the highest values of phenolic content was Ethanol:H_20 (50:50 v/v) and Ethanol:H_20 (75:25 v/v). In the case of antioxidant capacity for the majority of cases, Ethanol:H_20 (50:50 v/v) was the best. Furthermore, 100% H_2O and 100% ethanol showed to be the least efficient solvents in the extraction of phenolic compounds. Additionally, grape stems revealed higher values of these parameters, followed by grape pomace, and vine shoots with few exceptions.

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Modification of carob powder (*Ceratonia siliqua L.*) for by-product valorization in the food industries

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Carob fruit originates from the carob tree, is a leguminous evergreen tree (*Ceratonia siliqua L.*) cultivated in the Mediterranean area, namely in Portugal [1]. Its production is highly relevant, extensive, and sustainable. Carob pods contain various relevant nutrients, which include proteins, carbohydrates, and fibers [2].

In this study, after applying an innovative milling process to obtain an integral carob flour [3], carob powder with granulometry >100 µm, which is composed principally of insoluble fiber, was modified by a two-step acidic/thermal and enzymatic hydrolysis to improve its functional properties as a result of the cleavage and degradation of cellulose, hemicellulose, and lignin compounds [4],[5]. The optimal time and solvent concentration were considered as experimental factors for the acidic/thermal hydrolysis, as well as the ratio of enzyme-substrate for the enzymatic hydrolysis. The selected response variable was the solubility. The assessed solvents were sulfuric, lactic, and acetic acids, deionized water at 100 °C, and the enzymes were cellulase and xylanase. Furthermore, it was assessed how the steps could be minimized in order to decrease process's costs and runtime at industrial level.
The acidic/thermal hydrolysis results show that the lower solvent concentration and the higher hydrolysis time were optimal conditions for increasing the solubility. However, using an acidic solvent did not differ significantly from using deionized water, a greener and more environmentally solvent for industrial scales. As for the enzymatic hydrolysis, results suggest that when applied as an individual step, it could improve the solubility by 30%, but when applied uninterruptedly after the thermal hydrolysis, no significant improvement in solubility was detected. In conclusion, the optimal modification process for carob powder encompasses a single thermal hydrolysis using water to obtain 50% solubility of the initial carob powder. The resulting liquid by-product can be used to produce sweeteners, fillings, creams, etc., highlighting the importance of food-chain valorization in food industries.

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[S&EC 33]

Polysaccharides from orange peels: from waste to possible astringency modulators

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Polyphenols have been widely studied for their potential benefits to human health.[1] These compounds are essentially found in plant-based products, contributing to their organoleptic properties, namely color and taste (astringency and bitterness).[2] The main characteristic of polyphenols is their ability to interact with proteins, namely salivary proteins (SP), a phenomenon that is at the origin of the astringency sensation. Astringency is defined as dryness, tightening, and puckering sensations perceived in the oral cavity during the intake of astringent compounds. Furthermore, a balanced level of astringency can be considered a good quality parameter, such as red wine, but when it is perceived at high levels can lead to consumer´s rejection.[3]
Currently, polysaccharides have been described as able to modulate astringency, as they may reduce or inhibit the interactions between SP and polyphenols.[4-7] Vegetable and fruits processing generates a large amount of wastes (e.g., peels and seeds), which are often rich in bioactive compounds, such as polysaccharides.
The main aim of this work was to evaluate the inhibitory effect of polysaccharides, extracted from orange peels, on the interaction between SP and different classes of polyphenols.
So, the work was divided into two steps: 1) Isolation and characterization of different fractions of pectic polysaccharides from orange peels by sequentially extraction with several solvents (water, imidazole and sodium carbonate solutions); 2) Characterization of the SP profile before and after interaction of different polyphenol classes by HPCL and SDS-PAGE; 3) Characterization of the SP profile of the previously interactions in the presence of the polysaccharide fractions by SDS-PAGE.
The results showed that most of the polysaccharide fractions were effective in inhibiting polyphenol- protein aggregation and precipitation, mainly the ones extracted with imidazole and sodium carbonate. Considering the SP families, PRPs and cystatins were those in which it was observed less precipitation in the presence of polysaccharides.

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[S&EC 34]

Repurposing sardine cooking wastewaters as ingredients for European seabass diets

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There is a tendency to replace marine ingredients in aquafeeds with more sustainable vegetable protein sources. However, this often reduces feed intake, growth and alters flesh quality. This work evaluated aromatic extracts from sardine cooking wastewaters, a canning industry by-product, as intake stimulants in highly vegetable diets for European seabass (*Dicentrarchus labrax*), along with assessing possible impacts on organoleptic characteristics.

Sardine cooking wastewaters were either used directly (CW) or after processing by vacuum distillation (VD) or liquid/liquid extraction with soybean oil (LLE). The extracts' chemical profile differed, but the most abundant compound in all was the 1-penten-3-ol, hence selected as marker and included at 2 µg/g in plant protein-based diets. Thus, four isolipidic and isoproteic diets (one for each extract and a non-supplemented control) were produced and assigned to triplicate groups of fish (initial weight 95.7 ± 13.5 g), hand-fed twice daily until apparent satiation. After 18 weeks, fish growth performance was evaluated. Flesh colour and texture were assessed instrumentally and by sensory analysis with a consumer panel, using a Rate-all-that-apply methodology (RATA).

Fish fed LLE displayed a significantly higher feed intake than those fed CW, although neither differed from the control. LLE also increased feed conversion ratio, but final weight remained similar among diets. No differences were found in skin or muscle colour. Despite a lower fillet hardness in fish fed LLE compared to the control, no differences were perceived by the sensory panel; global liking was similar among treatments, being generally well accepted. Taste and odour of all samples was similar, with a "characteristic fish" and "soft" odour/taste. Overall, all tested diets yield fish with high quality for consumption and results suggest that aromas from sardine cooking wastewaters can modulate feed intake, but further optimization of processing and/or incorporation levels may potentiate their effectiveness on fish growth.

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[S&EC 35]

The use of subcritical water extraction for the recovery of protein from sardine residues

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Nowadays, due to the growing world population, we face sustainability issues caused by animal protein shortage and environmental impact of animal protein-based industries [1]. This rose the need of full usage of high-quality protein sources, capable of being produced on a commercial scale and minimizing environmental impact. The design of extraction methodologies to recover protein from underused biomasses, using sustainable approaches, emerges to create value-added ingredients for food and feed applications, easing these sustainability problems.

The present work aimed to recover and solubilize protein-rich fractions from sardine heads residues, using subcritical water extraction. Extractions were performed at temperatures ranging from 150 to 250 ºC. Further, a sequential treatment with two steps was also tested: the first one at 120 ºC, to recover soluble protein, followed by a second one at 180 ºC, to hydrolyse and solubilise the remaining insoluble protein. The protein fractions were analysed for their lipid, protein and ash contents, and their potential antioxidant activity with colorimetric assays.

For protein quantification, sardine residues' samples were freeze-dried and analysed by Kjeldahl and the protein solubilized fractions were analysed by Bradford. The ash content was obtained with a muffle furnace and the lipid content by the Bligh and Dyer method.

The protein solubilization of the fractions and their protein content increased with the increase of temperature. Moreover, the colorimetric assays revealed that all samples presented antioxidant activities, being the 250 ºC sample the one presenting the highest antioxidant activity. Further, the sequential process allowed the recovery of two fractions with different functionalities.

Taken together, the use of subcritical water extraction has proved to be an efficient and sustainable method to recover and solubilize protein from sardine residues, and that these residues have great potential to be included in food and feed formulation as a protein-rich and bioactive fraction.

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Production of bioactive protein-enriched hydrolysates from fish by-products using autohydrolysis

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Protein demand is expected to double by 2050 [1]. The amount of by-products generated from the fish processing industry represents more than half of the entire fish weight. These materials are commonly discarded with high costs and environmental burden [1,2]. Giving their high protein content (57%, dry weight), fish- based by- and co-products could be used in the development and fortification of food and feed products, thus contributing to bioeconomy and sustainability. Hydrolysis has been widely used to extract protein from several matrixes and the resulting hydrolysates usually exhibit interesting bioactive and functional properties [3,4].

The present work aimed the production of bioactive protein-enriched hydrolysates from fish by-products, using water under subcritical conditions. For that, the raw material was homogenized and centrifugated at 8000 g and 4 ºC for 30 min. The resulting solid biomass was used for the hydrolysis experiments. Several temperatures (150 to 230 ºC) and times (5 to 30 min) were tested, using 20 g (dry weight) of biomass and 0.4 L of water. After the hydrolysis, the solubilization yields were determined and the hydrolysates were analysed for their chemical composition, peptide profiles (HPLC) and antioxidant activity (in vitro colorimetric assays).

The solubilization yields increased with temperature and time of reaction. All hydrolysates presented antioxidant activity, being the best conditions at temperatures between 170 and 230 ˚C. Moreover, all hydrolysates presented high protein content (up to 82%). Autohydrolysis proved to be an efficient method for the solubilization and hydrolysis of protein from fish by-products without the need for chemicals or expensive enzymes. Antioxidant hydrolysates with high protein content were produced, which could be useful in the development of and fortification of food and feed products and could be a possible path to a rational and integral use of scarce protein sources in the near future.

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[S&EC 37]

Microalgae-biobased films for the poultry meat industry

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Poultry meat is gaining a significant presence in the population's diet. The worldwide trend of poultry meat consumption has indicated an increase in its consumption in detriment of beef meat. Since poultry meat is commonly marketed in the form of hamburgers, in group packaging of several units (4-6) separated by a plastic film, this study aimed to evaluate the potential of using a biobased plastic to replace these films and to analyse the potential bioactivity of those films to increase the shelf life of poultry meat. To reach this aim, chitosan (1.5% (w/v)) films, incorporated with 2% (w/w chitosan) of lignin to make them mechanically more resistant, and different levels of different microalgae (0.5%, 1% or 2%, w/w chitosan), were produced and used to separate the poultry meat burgers. The meat was stored at refrigerated temperatures, and the effect of these films on the quality of the meat (physicochemical characteristics: moisture, color, pH, total titratable acidity and lipid oxidation; and microbial contamination: mesophilic microorganisms, Enterobacteriaceae and psychrotrophic microorganisms) were evaluated. The films proved to be effective, by allowing a clear separation among the burgers, and the green colour of the films allows to easily differentiate the film from the burgers. In addition, its use to separate the burgers will not represent a hazard if ingested (contrary to fossil-based plastics), once the components are edible. The application of these films also provides an increased preservation of the poultry meat, compared to traditional plastics. Indeed, a lower microbial growth was observed with the biobased films, compared with the traditional films (2 log difference). Yet, the biobased films did not reduced the lipid oxidation. A greater integrity over time was observed in the biobased films due to the incorporation of lignin. The incorporation of microalgae to the biobased films did not improve the antioxidant and antimicrobial activity of the polymers, but changed significantly their colour, providing and easy and sustainable solution to substitute traditional plastics.

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[S&EC 38]

Walnut husk residues based-solutions for plant-parasitic nematodes management

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To protect crops from plant-parasitic nematodes (PPN), farmers resorts to plant protection products (PPP). Different PPP have been developed and used intensively to manage PPN. Nevertheless, due to concerns about impacts of PPP on human health and environment, many of them are being banned from the market and increased the need to gradually reduce the reliance on synthetic chemical pesticides and search for bionematicides. Among PPN, root knot nematodes (RKN, *Meloidogyne* spp.) are on top 10 PPN with major impact in agricultural areas [1]. In recent years, our team proved that walnut extracts and naphthoquinones, from walnut processing residues, have nematicidal activity against RKN, affecting mortality, hatching, penetration and reproduction, and is a very promising and attractive alternative to the use of synthetic nematicides [2,3]. As a result of the studies carried out, currently, the effect of walnut residues on the management of RKN are being evaluated. Preliminary results showed that *Meloidogyne* sp. density in soil, penetration and reproduction decreased significantly (61%, 68% and 65%, respectively), after soil treatment with walnut residues (pot assay). Additionally, fully degradable-in-soil mulch-based bionematicide delivery systems (BDS), comprising naphthoquinones-loaded biodegradable polymers-based mulches impregnated/coated with low-cost biopolymer-based formulations, were set up to protect the plant root system at transplanting and development. *In vitro* release of bionematicides from BDS, in water, revealed a negative effect on RKN mortality. Thus, the agro-industrial walnut residues can be valorised as renewable sources of naphthoquinones and potentially employed as bionematicides against RKN, when applied directly in the soil at pre-planting. These sustainable alternatives to nematicides to protect crops from RKN can contribute to improve crop production and protection systems of valuable crops, and also to promote circular bioeconomy. Part of the research work was developed in collaboration with Nogam.

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[S&EC 39]

Leaves blueberry's extracts as Valuable Ingredients in Cosmetic and Dermatological Products

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Blueberries are popular around the world due to their flavorful fruit and abundant bioactive compounds. However, there are large amounts of blueberry leaves being discarded following pruning in many countries. Exploring the bioactive compounds and application for these waste leaves would be strongly beneficial to the related agricultural industry [1]. Therefore, this study aimed to assess the viability of employing this byproduct in the prevention and treatment of skin issues. The antioxidant, antiaging, and lightening properties of aqueous extracts from blueberry leaves were assessed. Qualitative and quantitative evaluation of the extract's chemical composition was performed by HPLC-MS. The total polyphenol content (TPC) of biologically active compounds, such as the total content of polyphenols (TPC), flavonoids (TFC), as well as selected phenolic acids, were also evaluated.
Antiaging and lightening properties achieve 100% inhibition of hyaluronidase activity, 55.3 ± 4.2% of elastase, and 61.2 ± 10.5% of tyrosinase.
The obtained results indicate that blueberry leaves may be an interesting byproduct to be applied as a component of cosmetic and dermatological preparations with antioxidant, antiaging, and lightening properties

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[S&EC 40]

Antimicrobial activity of blueberry leaves against multidrug-resistant *Klebsiella pneumoniae*

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Klebsiella pneumoniae is a leading cause of nosocomial infections and is responsible for 10% of all hospital-acquired infections belonging to the critical and high WHO global priority list of antibiotic-resistant bacteria. Infections caused by K. pneumoniae are often severe and life-threatening since there is a high frequency of antibiotic resistance resulting in severe adverse outcomes. Consequently, there is an urgent need for new drugs effective against K. pneumoniae infections. One promising strategy is testing the potential antibacterial properties of natural resources, such as byproducts containing substantial amounts of bioactive chemicals, particularly phenolic compounds [1]. Therefore, the present study aimed to evaluate aqueous extracts of leaves blueberry in inhibiting the multidrug-resistant K. pneumoniae isolated from clinical wound infections. The phytochemical composition of these extracts was also determined and revealed that caffeoylquinic acid dimer was the major compound identified, corresponding to 46.0±0.4 mg/g. The results also noticed a positive relation between phenolic compounds content and antimicrobial capacity of the leaves blueberry's extracts. The extracts showed antibacterial activity against all K. pneumoniae strains isolated from clinical isolates with inhibition rate percentages equal to or greater than 50%. These data suggest that leaves blueberry's extracts aqueous extract may represent a valuable therapeutic source of compounds to be used against multi-resistant bacteria.

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[S&EC 41]

Antimicrobial activity against enteric bacterial pathogens of five natural mineral waters from the North of Portugal area

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The medicinal properties of natural mineral waters have been empirically known for centuries. Their use in the treatment of gastrointestinal, respiratory, skin, and inflammatory ailments is well documented [1,2]. Specifically regarding gastrointestinal diseases, it is known that natural mineral waters may be used in the treatment of gastric dyspepsia [3], intestinal inflammation [4], among others. Besides this, it has been shown this type of water has shown antimicrobial properties against several bacterial pathogens [5]. However, to the best of our knowledge, there are no studies regarding the antimicrobial effect of natural mineral water against gastrointestinal pathogens.

In this work, we have used five different natural mineral waters (NMW1 to NMW5) from different locations in the North of Portugal area and tested their antimicrobial activity against four pathogenic bacteria which commonly cause gastrointestinal diseases, namely *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella Typhi* and *Listeria monocytogenes*. For this, all bacterial strains were grown in a liquid medium prepared separately with each one of the natural mineral waters under study. The four bacterial strains and respective controls were incubated in 96-well plates at 37 °C for 24 hours under agitation, and their optical density at 600 nm was measured in order to determine the percentage of growth inhibition caused by the antimicrobial effect of the tested natural mineral waters.

The results obtained from this study have shown that the five natural mineral waters under study have antimicrobial properties against all the bacterial strains tested in the planktonic state, with a percentage of growth inhibition between 30% and 50%. Hence, it can be concluded that these waters may eventually be used as an aid for the treatment of gastrointestinal diseases caused by the pathogenic agents studied in this work.

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[S&EC 42]

Biodegradable bio-based mulching films derived from Cynara cardunculus (Cynara Mulch)

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Agricultural plastic mulch films are used in production of specialty crops to modify soil temperatures, conserve moisture, and reduce weed pressure, improving crop productivity. About 83 kton of mulch films were sold in Europe in 2019. Mulch films made from low density polyethylene (LDPE) are by far the most used currently. Recycling rates for mulches are substantially low, estimated below 30%. Portugal agriculture plastics demand in 2019 rounded 70kton. Plastic fragments and compounds released from plastic wastes are ubiquitous all over the world, and can be found in the atmosphere, water resources, as well as into soils (4 to 23 fold higher than what is released into marine environments) and organisms, including humans.

Biodegradable plastic mulches (BDM) have emerged as a promising alternative to LDPE pollution. BDM, made of different polymers, are designed to biodegrade in situ, into the agricultural soil. Their use may entail environmental impacts for the agricultural system that deserve to be explored on the short and long-term. New biomaterials represent a strategic approach for limiting environmental concern. Biodegradable components of lignocellulosic biomass are of particular importance for biopolymers development. Cynara cardunculus (Cc), an industrial non-food crop, with a residue biomass production that can reach 20 ton dried weight/ha presents its lignocellulosic fractions still widely unexplored, with its aerial parts presenting 53.0% polysaccharides (~10 ton/ha). With more than 100 ha installed just in Portugal, Cc presents the requirements to a sustainable biorefinery concept leading to the production of biobased ingredients with wide range of potential applications, such as biopolymers for agriculture, on a bioeconomy concept.

Process development for BDM films adapted to different crops needs and derived from lignocellulosic biomass, will offer an innovative, and environmentally friend approach to Cc exploitation towards biobased circular economy, potentiating a decrease of global plastic pollution, towards a sustainable environment associated to rural areas economic growth.

[S&EC 43]

Screening of enzymatic hydrolysis of mixed fish by-products

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The fish supply chain produces large volumes of by-products and losses [1]. Fish by-products and losses are generated mainly during processing but also in retail [1, 2]. Fish by-products and losses are protein-rich raw materials with bioactive and functional properties with recognised potential. The interest in protein hydrolysates has been rising as promising nutraceuticals with interest in animal health, including pet food markets [3, 4].

The present work aimed to perform an initial investigation (screening) of mixed fish by-product hydrolysate via single-factor analysis using a range of processing times from 1 to 6 hours and concentrations of Alcalase® from 0.5 to 3.0%. The hydrolysis involved using a ratio of 1:2 of 20 mM phosphate buffer at a pH of 8.0 prior to incubation in an orbital at 50 ºC operating at 150 rpm. The enzyme inactivation was performed at the end of each assay by heating in a water bath (95 ºC) for 10 minutes, followed by centrifugation. The supernatant was recovered and stored under refrigerated (-20 ºC) until analysis. The yield, hydrolysis degree (TNBS), peptide profiles (HPLC), soluble protein (BSA), and antioxidant and anti-hypertensive activity (in vitro colourimetric assays) were analysed.

The yield and hydrolysis degree increased with temperature and time of reaction until 6 hours. HPLC validates the formation of low molecular peptides. A more significant influence of processing time was verified to antioxidant activity (ABTS), with the best results at 3 hours. On the other hand, anti-hypertensive activity increased with enzyme concentration, namely using 1-3% enzyme at 3 and 4 hours. The optimal enzyme concentration and hydrolysis time were 3% and 3 hours, respectively. Antioxidant and anti-hypertensive hydrolysates were produced, which have the potential to support animal well-being and be used as nutraceuticals to prevent and relieve symptoms of several chronic diseases.

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[S&EC 44]

Industrial fruits by-products: determination of potential chemical contaminants

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Fruits like cherries, dates, grapes, and citrus, are consumed daily. Great quantities of agri-food residues are produced as a consequence of industrial processing. Those by-products have an excellent amount of bioactive compounds and have been reused in foods [1]. However chemical hazards can occur, such as mycotoxins, residues of pesticides or plant toxins, compromising their safety [1].

This study evaluated the presence of mycotoxins, residues of pesticides and tropane alkaloids by three different methodologies based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). Mycotoxins and tropane alkaloids were determined by Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC-ToF-MS) [2], while pesticides residues were determined by Liquid Chromatography coupled with QTRAP 5500+ MS/MS detector [3].

Regarding **mycotoxins**, ochratoxin A was only identified in one date seed sample from the Alig variety but at a level lower than the limit of quantification (1.5 µg/Kg). No other mycotoxins included in the method (aflatoxins – AFB_1, AFB_2, AFG_1 and AFG_2; zearalenone; toxin T-2 and fumonisins - FB_1 and FB_2) were found at levels higher than the limit of detection. No **tropane alkaloids** were identified in fruits by-products. Concerning **pesticide residues**, the grape seed sample had the highest number of residues detected (10), although lower than the maximum residue levels established for the edible part of the grapes. Orange, lime, and cherries presented at least one residue of pesticide above the MRL. None of the 155 pesticide residues tested positive in date pits.

These results showed that by-products could be contaminated with chemical hazards, especially pesticide residues. Most of the current research prepares extracts from food by-products to concentrate the bioactive components. However, this procedure increases/concentrates the content of contaminants. Thus, safety studies should be carried out in food grade extracts obtained from food by-products, to ensure that the valorization of these into new food products do not threat Human Health.

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[S&EC 45]

Industrial fruits by-products: assessment of the antioxidant capacity and determination of the phenolic profile

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Fruits are important crops in the Mediterranean area, well-known for their high biological activities. As consequence, several by-products, like kernels, stems, seeds, and peels from cherries, dates, grapes, and citrus fruits are produced every year [1]. This study assessed the antioxidant potential of these by-products, which have little current commercial use, although their considerable levels of bioactive compounds.

The by-products were collected from different food industries from Italy, Tunisia, Portugal, and France. For the characterization of the antioxidant capacity, ethanolic concentrated extracts were prepared [2]. The extracts were also tested for their antioxidant capacity using the β-carotene bleaching assay and the DPPH radical scavenging assay, as previously describe [2]. The Total Phenolics Content (TPC) and Total Flavonoids Content (TFC) were determined by Folin–Ciocâlteu reagent assay and aluminum chloride method, respectively. Also, the main phenolic compounds of fruits by-products were determined by Ultra-High Performance Liquid Chromatography coupled with Time-of-Flight Mass spectrometry detector (UHPLC-ToF-MS).

Among the assessed by-products, all three varieties of date seed extracts presented the highest TPC, TFC and antioxidant capacity. Epicatechin and catechin were the major phenolic compounds in date seeds, known for their antioxidant properties. Moreover, lemon pomace also showed a great antioxidant capacity, being rich in eriocitrin, naringenin and hesperidin. Also, phloridzin, a common phenolic compound in apples, was present in lemon by-product.

In general, all the studied fruit by-products showed good antioxidant capacity. These results support the valorization of these Mediterranean fruits by-products as a potential low-cost source of natural antioxidants, that could be used to prepare functional foods, helping to improve Human

Health, while reducing the environmental issue of food waste, and promoting a circular economy transition.

Acknowledgements: This work was financially supported by the research project ValICET (PRIMA/0001/2020) - Valorise foods and Improve Competitiveness through Emerging Technologies applied to food by-products within the circular economy framework (section 2 PRIMA project) funded in Portugal by Foundation for Science and Technology (FCT). The work was supported by UIDB/00211/2020 with funding from FCT/MCTES through national funds. A.R.S.M. would like to thank to ValICET project for her fellowship (03/2022/BI). A. Sanches Silva is grateful to the Foundation for Science and Technology (FCT) for funding the ValICET project in Portugal (PRIMA/0001/2020). R Sendón is grateful to the Spanish Ministry of Science and Innovation and the State Research Agency (AEI) for funding the ValICET project (PCI2021-121965).

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Supercritical extraction as a new approach in the biovalorisation of agricultural residues

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The world's population, determined by the United Nations, is currently around 8 billion people, with an expected growth of another 2 billion by the end of 2100 [1], and it will be necessary to increase approximately 80% of world food production to meet the population's needs [2]. The result of this increase in production is the intensification of food waste and loss, which negatively affects the environment [3,4]. Worldwide, the rate of wasted food reaches 17%. Roots, fruits, vegetables and oilseeds are about 40 – 50% of this total [3]. Therefore, many studies have been conducted to find methodologies capable of adding commercial value to waste products [5]. Residues and by-products have several bioactive ingredients, such as phenolic compounds, carotenoids and vitamins [4,6].

The present work aims to evaluate the bioactivity of extracts using olive pomace, olive leaf, white onion and collard as raw materials and optimize the extraction conditions using SFE-CO_2 (supercritical fluid extraction using carbon dioxide) - a sustainable and green technology. These products are rich in bioactive compounds with functional properties. The chemical composition of the obtained extracts was evaluated by liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), and their toxicity was evaluated through a non-tumour cell line. Extractions were carried out using a 1L extractor with a pressure range of 80, 90 and 100 bar, a temperature of 50 °C and two hours of extraction time. In general, higher pressures have allowed higher extraction yields. Concerning the chemical composition, GC-MS analysis revealed the presence of palmitic acid, linolenic acid, linoleic acid, stearic acid, oleic acid, squalene and vitamin E.

In the future, it is expected to incorporate these bio-compounds into fruit and vegetable products such as juices, jams, and fruit purees.

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