Dare 2 Change

INNOVATION DRIVEN AGRIFOOD BUSINESS

Abstract Book

18-19 NOV 2021
Centro de Congressos
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PORTUGAL FOODS
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COLAB 4FOOD

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Instituto Nacional de Investigação Agrária e Veterinária, I.P.

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Dear colleagues,

It was with great pleasure that we welcomed you in Porto, for the first Dare2Change conference.

This scientific conference was the joint effort of three institutions - Colab4Food, INIAV and PortugalFoods - to bring a technical-scientific and business oriented overview of the agri-food sector and the relationships between academia and industry.

The COVID-19 pandemic has brought greater urgency to the Agri-Food sector's response to pressing issues for society and for its own competitiveness. In parallel, the sector aims to guarantee the sustainability of its processes and value chain, ensuring efficiency gains, while reducing waste and the ecological footprint of its products, while changing business models in the face of the disruption imposed by digitalization and public policies. On the other hand, it has to face the growing concerns and focus of consumers on issues related to health and wellness, anticipating trends and creating the diets and nutrition of the future.

With this *Book of Abstracts,* developed with the contributions submitted and approved for poster communication by the Scientific Committee, we hope to take the scope of this event beyond the limits of its physical occurrence.
This issue contains 90 abstracts presented at this 2021 edition, divided in the following five themes: Digitalization & Mechanisation, Food Development & Production, Food & Consumer Trends, Health & Wellbeing and Sustainability.

We hope you benefit from this editorial work and that it encourages you to bring your work to the future edition of Dare2Change!

The Organizing Committee,

MIGUEL TEIXEIRA
Scientific & Executive Director | Colab4Food

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This publication brings together the abstracts of the poster communications presented at the Dare2Change 2021. All abstracts were evaluated by the Scientific Committee of the Conference.

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PROGRAM

Dare2Change Science 18.NOV

08h30 Reception

09h00 Opening Session

Nuno Canada, President INAV, I.P.

José Teixeira, Vice President - Colab4Food

Session 1 Sustainability

Moderators: Carina Almeida (INIAV); Jorge Noro (UCoimbra)

09h20 **Plenary Session** | Food Systems Innovation: A Force of Good for People, Planet and Prosperity

Andy Zynga, CEO EIT Foods

10h05 Towards the sustainability of the food system

Jesus Simal-Gandara, Universidade de Vigo

10h25 **Dare2Snack**

Session 2 Food Development and Production

Moderators: Manuela Vaz Velho (IPVC), Ana Sanches Silva (INIAV)

10h55 Bionanocomposites for Food Packaging - Challenges to Overcome, Opportunities to Explore

Ana Luísa Fernando, FCT, Universidade NOVA de Lisboa

11h15 FODIAC and TrustEat! Projects: Facing Health, Ageing and Digitalization in the future Food System

Lorenzo Pastrana, President of the Research Office and Principal Investigator of the Food Processing and Nutrition Group of the International Iberian Nanotechnology Laboratory

Session 3 Health and WellBeing

Moderators: Victor Freitas (REQUIMTE), Isabel Ferreira (UPorto)

11h35 Agrofood byproducts as sources of bioactive compounds for nutrition and health

Manuela Pintado, Director of CBQF CBQF-Center of Biotechnology and Fine Chemistry School - Escola Superior de Biotecnologia Universidade Católica Portuguesa

11h55 Diet and Health: Nutrigenetic and Nutrigenomic

Marta Silvestre, NOVA Medical School - Universidade NOVA de Lisboa

12h15 **Dare2Lunch**

Session 4 Food & Consumer Trends

Moderators: Anabela Raymundo (ULisboa), Deolinda Silva (PortugalFoods)

13h45 **Plenary Session** | Food & Agri: *quo vadis*?

Kees de Gooijer, Chairman of the Board of Directors, Topconsortium for Knowledge and Innovation for the Biobased Economy (TKI-BBE) and Director of the TKI Agri & Food

14h30 Balancing fear and freedom: the coming of age of the homebody food consumer

Ana Isabel Costa, CATÓLICA-LISBON School of Business & Economics, UCP

14h50 Going beyond liking: advances in the emo-sensory evaluation of foods

Luís Cunha, Universidade do Porto

Session 5 Digitization & Mechanization

Moderators: José Teixeira (UMinho), Miguel Teixeira (Colab4Food)

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António Camara, Universidade Nova de Lisboa

15h30 A Portuguese example on how digitized food quality and supply chains can support localized food production and augment consumer trust

Henrik Stamm Kristensen, Blendhub, Portable Powder Blending and Chemometric Brain

15h50 **Dare2Snack**

16h20 Ideas4Sharks

17h30 Closing Session

Maria do Céu Antunes, Minister of Agriculture

Dare2Change Business 19.NOV

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Frederico Santos, Head of Digital and Innovation, SONAE MC
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Edoardo Erlini, Sales & Special Project Manager. EZ Lab
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Rick Miller, Associate Director, Specialised Nutrition, Mintel
11h30 Upcycling for the future
Marcio Barradas, CEO, Essence Food
11h45 Solar Foods - Food out of thin air
Shilei Zhang, Chief Commercial Officer, Solar Foods
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14h15 Brands for Portugal
Carlos Coelho, Brand Specialist and IVITY Brand Corp President
14h30 Belong!
Joah Santos, Founder, NYLON
14h45 Wines of Portugal brand strategy
Sónia Vieira, Marketing Director - ViniPortugal
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Marketing and branding's future
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Bruno Rio (SONAE MC)
João Paulo Rocha (CEREALIS)
Nuno Bernardo (Super Bock Group)
Vitor Hugo Gonçalves (Sociedade da Água de Monchique)
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Deolinda Silva, PortugalFoods
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Abstracts of Poster Presentations

Dare 2 Change

Digitalization & Mechanisation

[DME 1]

Artificial neural networks vs partial least squares modelling for rice quality prediction based on NIR spectroscopy

Pedro Sousa Sampaio[1,2,3,*], Carla Brites[1,3]
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The different market valorization of rice (*Oryza sativa* L.) production urges for the continuous control of its quality, authentication, or contamination issues. Rice quality can be evaluated from grain physical parameters, as well as the milling performance, biochemical composition, and cooking properties [1]. The fast and accurate experimental data are essential for continuous and detailed information. The near-infrared spectroscopy and machine learning methodologies such as partial least squares (PLS) and Artificial Neural Network (ANN) present significative advantages for detailed prediction of rice quality parameters that are essential for consumers and industrial players. The advantages of ANN are related to the ability to learn based on examples, fault tolerance, operation in a real-time environment, forecasting non-linear data, and their superior prediction characteristics, making it a widely used statistical tool [2].
The parameters selected for rice quality prediction are related to biochemical composition (starch, amylose, ash, fat, and protein concentration) and pasting profiles (peak viscosity, through, breakdown, final viscosity, and setback). The prediction models based on PLS and back-propagation network (ANN) algorithms, used NIR spectra previously processed using specific algorithms and are characterized by significant coefficient determination for different pasting parameters such as breakdown (R^2=0.97; 0.99), through (R^2=0.96; 0.99), setback (R^2=0.97; 0.99), peak-viscosity (R^2=0.97; 0.99), and final viscosity (R^2=0.95; 0.96).
The models obtained based on ANN algorithms are considered as a valuable tool to predict several rice quality parameters in fast and clean mode, that are considered fundamental to industry and consumers, allowing to assess its cooking behavior, providing an important contribution to the rice value chain, saving time and decreasing costs associated to the detailed analysis processes.

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Food Development & Production

[DPA 1]

Gluten-free bread enriched with microalgae biomass pre-treated with ethanol: improvement of bread quality, nutritional and sensory properties

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This research is included in the project "Algae to Future" that addresses the potential of microalgae as healthy ingredients for food in the future[1].The present study aims to compare the impact of adding 4% (w/w) raw and ethanol treated *Tetraselmis chuii*, *Chlorella vulgaris* and *Nannochloropsis gaditana* on dough structure and technological aptitude, nutritional composition, bioactivity and sensory acceptance of gluten-free breads based on buckwheat and rice flours.

There is a growing interest in using microalgae as a renewable and sustainable raw material, which can be used in different matrices[2] as a promising functional food source[3]. Their incorporation in food can lead to changes in the rheology, texture, nutritional composition, and sensory properties[4,5]. Therefore, microalgae biomasses were subjected to ethanol pre-treatment to remove some colour and flavour components, to contribute for the increase of consumer acceptance. Moreover, the development of gluten-free foods is still a challenge, and all the ingredients can play an important role to achieve good nutritional and sensory profile.

In this research, the technological aptitude of the gluten-free doughs enriched with microalgae biomass was studied, according to the rheological properties. Firmness, cohesiveness, volume, colour, nutritional and chemical composition of the breads were also evaluated. The bioactivity was evaluated by determination of the total phenolic compounds, antioxidant activity and pigments (chlorophyll-a, chlorophyll-b, and carotenoids). For the sensory analysis, only the control and breads with *Chlorella vulgaris* incorporation, treated and without ethanol treatment, were tested.

The obtained results evidence that breads with ethanol treated microalgae induced improvements in terms of bread texture, volume and sensory acceptance accompanied by an enriched nutritional composition. This finding indicates that ethanol treatment might be a viable strategy for producing microalgae biomass with an improved sensory profile, that can be used in different food stuffs, such as gluten-free bread.

Acknowledgments: The work was supported by the Norwegian Research Council, project Algae to Future - A2F (NFR 267872), and Portuguese Foundation for Science and Technology (FCT), through LEAF Research Center UIDB/04129/2020. We would like to thank Sónia Oliveira, Sheyma Khemiri, Joana Sales and Joana Soares for all the help during the practical work in the laboratory.

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[DPA 2]

Fermented food and microalgae as clean label ingredients in plant-based mayonnaises

Sara Simões[1*], Joel Santos[1], Diogo Castelo – Branco[2], Diogo Figueira[2], Ana Tasso[2], Catarina Prista[1], Anabela Raymundo[1]
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"Clean Label" is one of the strongest market trends in the food industry. Consumers are looking for simpler formulations, with less ingredients and easily recognizable origins. This trend ties with more recent tendencies, such as plant-based ingredients, "better for you" foods and eco-friendly products[1,2].
Fermented foods (FF) are known for their enhanced potential to improve the health of consumers[3] compared to their unfermented raw materials. Besides, their high content in organic acids and other antimicrobial metabolites produced by fermentative microorganisms makes them long shelf life products[4].
Microalgae are raw-materials with high levels of protein (up to 70% in mass) and bioactive compounds, molecules of great nutritional interest[5]. *Chlorella vulgaris* has been successfully incorporated in food emulsions, conferring colour and antioxidant capacity[6]. The combination of the nutritional and technical impact of this ingredient might positively influence human nutrition and product formulations.
As part of the cLabel+ project, this work aimed to evaluate the potential of FF such as green pea tempeh flour and chickpea tempeh flour, grass pea sweet miso, and "Honey" Chlorella spp. from Allmicroalgae, as natural preservatives in plant-based Mendes Gonçalves' mayonnaises, to obtain clean label products.
Green pea and chickpea tempehs were obtained by standard processes. After fermentation, the blocks of tempeh were cut in 0.5 cm slices, and dehydrated for 3 h in a convection oven at 95 °C. After, completely dried and cooled tempehs were milled using a Variable Speed Rotor Mill Pulverisette 14 premium line (Fritsch) until a 35 mesh flour was obtained. Grass pea sweet miso was obtained as described in Santos et al. (2020)[7].
FF and "Honey" *Chlorella spp*. were submitted to chemical and nutritional analysis, and their antimicrobial activity determined by Disk Diffusion Test. From the results obtained, it was possible to conclude that these products can be used as alternatives to traditional preservatives, resulting in clean label products with improved nutritional characteristics than the original mayonnaises.

Acknowledgments: We want to thank Marta Cabrita for the optimization of plant-based mayonnaises. We also want to thank the funding from PO CI/ANI/ 46080/2019 cLabel+: "Alimentos inovadores "clean label" naturais, nutritivos e orientados para o consumidor" and from FCT, research unit UID/AGR/04129/2013 (LEAF).

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[DPA 3]

Comparative analysis of resistome and mobilome of oyster aquacultures with other aquatic environments

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Shellfish farms are often located in rivers and estuaries, near urban areas which are generally considered to be natural reservoirs of antibiotic resistance genes (ARGs) leading to antibiotic resistant bacteria entering the food chain [1]. These ARGs can also be transmitted to terrestrial animals and the environment, as an example of the One Health concept. On the other hand, the aquaculture industry also contributes to the increasing number of ARGs in aquatic environments. Against this background, we aimed to conduct a comparative analysis of the profile of ARGs in semi-intensive aquaculture farms with other aquatic environments and to identify the epidemic potential of these antibiotic resistance genes in microbial communities. For this purpose, we developed algorithms for counting ARGs and mobile genetic elements (MGE) in metagenomes from selected aquatic environments.

Research: High-throughput sequencing-based metagenomics was used to characterize the wide-spectrum profile of ARGs in sediments from bivalve aquacultures and other aquatic environments. Analysis of sequences was performed using the MG-RAST pipeline. We searched for ARGs orthologues by sequence, classified into antibiotic resistance families, and for relaxases, transposases and integrases, indicative of MGEs.

Results: We characterize both the diversity of antibiotic resistance genes and estimate the number of mobile genetic elements in all microbiomes of our dataset. Our preliminary results show that the microbiomes of natural environments are very rich in ARG and MGE. Yet, the ratio of MGE/ARG is higher in estuarine aquacultures, suggesting that the capability of resistance determinants transfer is higher in these regions subjected to anthropogenic activities.

Conclusions: Although environmental microbiome samples from pristine environments such as Antarctica share a very rich and diverse repertoire of ARGs and a high number of horizontal gene transfer elements, river aquaculture microbiomes show a higher epidemic potential for spreading ARGs compromising the safety of these products for human consumption.

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[DPA 4]

A new way to consume Chitosan's impact in edible packaging

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Plastic packaging is one of the most serious issues because they are ubiquitous in our lives. Biodegradable plastics have been in the spotlight as an alternative solution, however they have their own limits, because they require time to completely degrade[3]. As a result, another alternative approach has emerged: edible plastic or using our own body has a biodegradable machine.
Chitosan may agglomerate the lipids around it, allowing the formation of a protective biofilm[1]. This technology has the same application as traditional plastic packaging, but the key point is that it may be edible to us. Indeed, it extends shelf life by minimizing the rate of respiration and reducing the water loss[2]. The second key point is that chitosan may be found in highly consumed food such as shrimp, crab, squid and so on. Chitosan is simply made by processing natural shrimp, crab, and squid waste. The process begins with grinding raw material, followed by demineralization, deproteinization and decolorization to obtain chitin, and lastly, the deacetylation, before obtaining chitosan powder[2]. Another point is that the external aspect of the food is very important to the consumer, thus, chitosan is absolutely undetectable by the consumer on their food and allows it to retain its external colour, making the food more attractive. The final part is Chitosan's anti-microbial properties; the coating generated by Chitosan creates a membrane disruption, a cell lysis of the bacteria. Therefore, the coated fruit is more likely to be consumed in a longer period of time.
In the light of those points, we can convincedly believe that Chitosan is a great answer since it's simple to produce, it fulfils all the needs of traditional plastic film, such as being antimicrobial, and it's edible and biodegradable. Therefore, it's a highly convincing answer in almost all aspects.

Acknowledgments: A. Pecot and H. Gomes

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[DPA 5]

Nano spray dryer as strategy for the production of sub-micro and micro sized sugar

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New trends in food industry have shown great interest in obtaining healthy products using clean-label ingredients. Among all food challenges one of the highest challenge is the reduction of sugar. One of the most studied alternatives are artificial sweeteners, in order to replace or reduce the amount of sugar in food formulations. However, sugar substitution decrease palatability and consumer acceptance of food products [1]. Therefore is important to study new strategies to reduce sugar in food formulation with lower impact in the food product and the added ingredients. The aim of this work was to reduce the particle size of sucrose in order to increase sweetness mouth sensation by increasing the surface area. Nano-Spray Drying (NSD) technology was used to produce the particles. To increase the stability of obtained powders different components were tested in combination with sucrose. Maltodextrin, inulin and octenyl succinic-modified starch at different concentrations were combined with 10% (w/v)) of sugar solution concentration and processed by NSD and evaluated regarding their water activity (Aw), moisture content, yield and particle size.

Results showed that drying of sugar without carrier had the lower yield (42%) compared with sugar powder dried with carriers (63-81%). The moisture content varied for the studied drying conditions, however all were kept below 5%. Aw remained below 0.4 for all tests, which indicates a good stability after drying process. Sugar powder obtained with different carriers had particles sizes between 3.1 and 4.8 µm, however, it was not possible to measure the particle size of the sugar powder without any type of carrier due to its high hygroscopicity [2]. Obtained results indicate the effectiveness of the carriers to increase the stability of the powders dried by NSD, however, further studies need to be performed in order to optimize operation conditions of drying process to obtain low particle sizes.

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[DPA 6]

By-products from the fruit production and macroalgae from the Portuguese coast: a sustainable partnership to develop nutritious snack balls

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Macroalgae are increasingly integrated in foods due to the health benefits they provide, resulting from the high number of bioactive compounds present in their composition, as well as the high content of polysaccharides and proteins1-2.
This study aimed to develop an innovative nutritious snack, incorporating three macroalgae from the Portuguese coast: *Porphyra dioica* and *Gracilaria gracilis* (red macroalgae) and *Ulva rigida* (green macroalgal), at different concentrations (1%, 3%, 5% and 10% w/w). The impact of the addition of macroalgae was studied in terms of TPA (Texture Profile Analysis) and rheology behaviour. A colorimeter was used for colour evaluation. Nutritional composition was evaluated by AOAC methods (lipids, ash, moisture) and protein content by DUMAs. Mineral profile, using ICP-OES, was also studied. Bioactivity was accessed by total phenolic compounds (*Folin-Ciocalteu*) and antioxidant activity (DPPH and FRAP assays). Sensory properties (colour, aroma, flavour, texture and general acceptance) were studied, as well as the maximum level of incorporation, to understand the acceptance of the algae in the product.
Healthy-balls are based on chestnut and apple flours, obtained from the by-products of small-calibre fruits that don't meet all the quality standards for market, contributing to the non-waste and sustainability of the product. The use of chestnut flour3 and psyllium4 promotes the strength and stability of the gel.
The texture results showed a decrease in the firmness of the gelled snack with *Ulva rigida* and *Porphyra dioica* addition, but for *Ulva rigida* this effect is significant ($p<0.05$) only with 5% and 10% incorporation. This wasn't observed for *Gracilaria gracilis*, attributed to its content in agar, a structuring polysaccharide[5].
The results showed that the three macroalgae can be used as innovative and sustainable ingredients to nutritionally enrich snacks using high levels of incorporation, with more nutritional claims, particularly in terms of mineral content.

Figure 1: Nutritious snack balls developed with different types of macroalgae (0%,1%, 3%, 5% and 10% w/w).

Keywords: Macroalgae, nutritiousballs, snack, texture, bioactivity, sensorial evaluation,.*Ulva rigida*, *Porphyra dioica*, *Gracilaria gracilis*

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[DPA 7]

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 new insights in the use of PC as emulsifier agents.

In this study, the molecular perspective of the use of PCs as emulsifiers has been studied in a yeast protein extract (YPE)[6]-based mayonnaise in comparison with the traditional egg derived mayonnaise Thus, the molecular mechanisms of the interaction between egg or YPE protein models and PCs (gallic acid-GA, tannic acid-TA, and grape seed extract-GSE) 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 (4 °C and RT) simulating the storage conditions.

Overall, different mechanisms of molecular interaction were found for the different PCs. Molecular affinity constants were calculated by using the Stern-Volmer equation. A generally trend to higher constant affinity was observed in YPE model when compared to egg proteins. Likewise, the GSE achieved the higher binding affinity constants followed by TA and GA. Overall, two main binding mechanisms were found in this study depending on the PC tested. The results obtained within this study clearly showed the potential of PC to be used as natural emulsifiers, 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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[DPA 8]

Use of autochthonous mesophilic lactic acid bacteria as starter cultures for ovine cheese production

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In this essay, the impact of using a mixed inoculum, consisting of two lactic acid bacteria isolated from aged Serpa PDO cheese and previously characterized for its technological, probiotic, and antimicrobial properties[1,2] was evaluated. For this purpose, a pilot scale trial was developed using a mixed inoculum consisting of the autochthonous strains *Lactobacillus paracasei* A2Lb1 and *Lactobacillus plantarum* G1Lb5. Three batches of cheese (performed in triplicate) were produced based on the Serpa cheese specification, except for the use of inoculum. In the first (F1), used as a control, only raw milk was used, in the second (F2) raw milk with inoculum and, finally, in the third (F3), pasteurized milk with inoculum. The cheeses obtained were evaluated in triplicate, immediately after production (0 days) and at 15 and 30 days of ripening, for the main physicochemical, microbiological (total mesophiles, enterobacteria, lactic bacteria and fungi) and sensory characteristics, using standard techniques.
The total and lactic acid bacteria counts were significantly higher at the end of ripening in inoculated cheeses, made with raw (F2) or pasteurized (F3), than in raw milk cheeses (F1). Added native starters appear to minimize growth of *Enterobacteriaceae* and *Staphylococci*, including positive coagulase ones, but slightly stimulate fungal (yeast) growth, with significantly different counts at the end of ripening, between different batches. In the physical-chemical parameters, significant differences were also observed between the three batches, after 15 days of ripening, highlighting the intense acidification of the inoculated cheeses. These characteristics were reflected in the sensory evaluation, where differences were detected in terms of color, texture and flavor, which were more accentuated in cheese made with pasteurized milk. These results reflect the need to optimize the starter in quantitative and qualitative terms, in order to minimize the differences between inoculated cheeses and the cheese obtained by the traditional process.

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[DPA 9]

Cultured Meat – An Overview

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The world population is growing and is expected to exceed 9.7 billion by 2050, which raises several concerns about lack of animal protein. Cultured meat, can be defined as artificial meat obtained from stem cells and their proliferation through cell engineering[1], and is seen as an alternative for consumers who do not wish to change the composition of their diet. However, some regulatory issues, as well as challenges from a technical and economic point of view, have hampered the positioning of cultured meat in the market. Some of the current work focuses i) an alternative to fetal bovine serum for the culture medium[2], ii) improving the nutritional and sensory aspects of the product, with efforts to develop cultivated fat as an additional ingredient[3], iii) scaffolding for obtaining of a 3D structure with great thickness and texture[4], and iv) use of computational modelling can accelerate process optimization and reduce the cost of transformation[5].
There have been numerous start-ups aimed at the development of cultured meat and its positioning in the market[6]. Investment in the nascent field exceeded 300 million € in 2020, nearly double the previous cumulative investment in the industry[6]. Singapore was the first country to authorize the sale of this products, with the chicken nugget obtained in cell culture, by the start-up Eat Just. Despite overcoming animal sacrifice, understanding and acceptance by the consumer is still far from happening. Marketing around cultured meat can have a strong impact on the perception of reality, making it important how the media portrays it[7]. The sustainability is under debate, and a careful assessment of the life cycle will be crucial to understand the true environmental impact. We intend to provide a general and recent overview on this topic, addressing pros and cons, as well as its positioning in the market.

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[DPA 10]

Brewer's spent yeast polysaccharides a competitive alternative to commercial emulsifiers

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The spent yeast from the brewing process is one of the largest by-products of the brewing industry. In response to the concept of circular economy, there is a need to value it as a co-product. Yeast cell wall polysaccharides are mainly composed of mannoproteins and glucans, which have a wide variety of applications, making them products with a high added value. In the food industry, one of their applications include the use as bio-emulsifiers [1,2,3].
In order to evaluate these polymers' potential as emulsifiers, brewer's spent yeast polysaccharides were tested by measuring the emulsifying capacity (EC) of each sample for one month. The EC was measured by adding the different samples to 4 mL of water and 6 mL of vegetable oil and shaking (30s, hand shaking or by Ultraturrax), resulting in a water in oil emulsion. During the one month, the volume of the emulsion, and the volume of the water and oil phases that had already been separated from the emulsion were measured, enabling the calculation of the EC percentage.
The results showed that the different soluble extracts have different performances: some have no EC; others have a good performance when applying low energy (hand shaking), while others needed high energy (Ultraturrax) to have EC. Nevertheless, the extract which contained mannoproteins with lower amount of carbohydrates, when used in a 2.0% (w/v) concentration at pH=11 (pH emulsion=5.23) and 0.5% of NaCl, when applying low energy has the best EC (85%) for one month, similar to the EC (83%) of the commercial emulsifier (Xanthan Gum), at the same conditions. However, this occurs when applying high energy, since low energy is not an option for Xanthan Gum.
Brewer's spent yeast polysaccharides seem to be a competitive alternative to the commercial emulsifiers, with promising uses in different foodstuffs.

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[DPA 11]

The impact of geographical and temporal factors on grapes' microbiota of the viticultural Nemea PDO zone of Greece

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Microbial terroir uncovers the impact of vineyard characteristics on grapes' microbiota suggesting that there is a unique microbial fingerprint associated with the terroir. Agiorgitiko (*Vitis vinifera* L. cv.) is the most popular indigenous grape variety in Greece, grown in the Protected Designation of Origin (PDO) Nemea zone. Both terrain and climate conditions are quite diverse along the zone, resulting the informal division of the zone into three sub-zones at altitudes ranging from 250 to 800 m.

The present study, the first of its kind in Greece, aims at depicting the microbial fingerprint of Nemea, by examining the microbial biodiversity of grapes and soil over time and space using amplicon-based metagenomics analysis. Towards this, 25 vineyards from five different areas of the Nemea PDO zone were selected, and grape and soil samples were collected at harvest from two consecutive years (2019 and 2020). Total DNA was extracted from all samples and the V1-V3 region of the 16S rRNA gene and the ITS1-TIS2 DNA region were sequenced and analysed by bioinformatics and statistical tools for the identification of the bacteria and yeasts/fungi diversity, respectively.

The results revealed that soil biodiversity was higher than that of grapes, which is in accordance with similar studies. Regarding the different factors affecting the microbial terroir, the harvest year and the region of origin within the zone were examined. Principal Coordinates analysis (PCoA) clearly revealed the discrimination of both grape and soil samples based on the harvest year. In the case of yeasts/fungi, although similar families were identified, the relative abundances of these families were different between the two years for both grape and soil samples. In the case of bacteria microbiota, a completely different microbial fingerprint was detected at the family level, both in soil and grape samples, between the two years. On the other hand, the geographical origin couldn't discriminate the samples both in 2019 and 2020, highlighting the joint microbial fingerprint of Nemea PDO zone.

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Design thinking for food product development: testing brainstorming techniques

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Design thinking (DT) is a user-centred approach that uses design tools and methods to solve non-linear problems. The DT method popularized by IDEO consists of a 3-spaces system: inspiration, ideation and implementation. Brainstorming is considered the best ideation method for DT; however, it can be supported by different design tools and techniques. This work aims to compare free-brainstorming with brainstorming guided by tools designed for the purpose in the context of rice-based food product development.

The problem framework was given by the results from a rice choice questionnaire and the physicochemical data collected on whole rice and rice bran. Two groups of students worked on the exercise: G1 was asked to work on the problem using free-brainstorming and to get three good ideas; G2 brainstorming was supported by a personas exercise (the creation of a hypothetical consumer based on a cluster analysis of the questionnaire data) and a design tool consisting of 109 cards with words generated by a free word association exercise performed by chefs. G2 was asked to pick three cards and write down the ideas that came to their mind; an hour later, they chose the recipes that best suited each persona. The ideas were evaluated by a jury of three chefs, regarding adequacy to the consumer, novelty, workability and clarity on a 4-point scale and compared through Mann-Whitney U-test.

G2 generated 82 ideas, from which the students considered 16. The results showed differences ($p<0.05$) regarding novelty and workability between groups: the three ideas from G1 received a lower novelty score than the ideas from G2; contrarily, G1 ideas showed higher values regarding workability; adequacy values didn't show any differences among groups, suggesting the personas exercise had no impact on the idea rating. The results show that the cards-tool helped produce ideas considered novel by the chefs.

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[DPA 13]

Food Labelling Handbook – An integrated approach from business operators to final consumers

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Today food information is a fundamental tool to enable consumers to make purchasing decisions that are informed and that ultimately reflect their concerns in terms of health, economic interests and ethical values.
Because of the role that food information plays in contemporary societies, over the last decades food labelling has been increasingly subject to regulation by public authorities both at international and national level. The European Union (EU) makes no exception in this respect: currently, the vast majority of food labelling requirements that businesses operating across the EU market must comply with are laid down in European directives or regulations.
Notwithstanding the extensive EU's regulatory intervention in this area, under certain conditions EU countries may still impose their own national labelling rules for specific product categories or aspects that are not subject to European harmonisation. In Portugal, for instance, national legislation regulates labelling requirements for several food categories (e.g. soft drinks, coffee, vinegar, etc.) and for the provision of origin information of milk, among others.
Therefore, the coexistence of European and national labelling requirements ultimately results in a complex web of legal obligations that, on the one hand, business operators are required to observe and, on the other, competent authorities must enforce to make sure that food trade takes place under fair conditions.
In light of the above, through the eyes of distinguished academics, professionals, representatives of the competent authorities and of the civil society, this handbook [1] provides the reader with the necessary tools to understand how food labelling is regulated, applied and used in Portugal, including from the perspective of businesses and final consumers. At the same time, it offers valuable insights into possible future developments in this area, notably with regard to front-of-pack nutrition labelling, country of origin information, labelling of alcoholic beverages and animal welfare labelling.

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[DPA 14]

Gracilaria verrucosa potential as a source of protein: assessmentusing the Osborne method

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Gracilaria verrucosa is a seaweed from the *Rhodophyta* filo that is widely used for commercial purposesregarding the production of agar aiming the application of its functional properties in food products [1]. However, in general, red algae species have higher and interesting protein contents when compared togreen, and brown species (ca. 47, 26 and 15% $(w/w)_{dw}$, respectively), that could confer techno- and bio-functional features and allows the substitution of traditional animal protein sources [2], [3].
Thus, the main aim of this work was the fractionation of proteins from *Gracilaria* using the Osborne method [4], which is based in the protein affinity for different solvents: dH_20, NaOH, NaCl, and EtOH. Each solvent was applied sequentially, and the residue was used for further extraction with the next solvent. Each extraction step was repeated twice to improve protein recovery. The protein content of each fraction was determined using the Lowry method and results were compared with the initial proteincontent present in the biomass which was previously determined using the Kjeldahl method.
Results showed a total yield of 46.6% $(w/w_{seaweed})$. *Gracilaria* has shown to be mainly composed by proteins that are soluble in water and NaOH (46.0 and 30.5% $w/w_{seaweed\ protein}$, respectively) and in minordegree by proteins that are soluble in NaCl and EtOH (10.6 and 12.5% $w/w_{seaweed\ protein}$, respectively). This was expected, as red seaweeds are known for the high content in phycobiliproteins, which are generally soluble in water. Regarding all the fractions, it was possible to recover 99.6% of the 15.7% of total protein content present in the initial biomass.
This study allowed to observed that *Gracilaria verrucosa* protein profile can be characterized from protein fractionation with encouraging yield, which can create new opportunities for future applications for food and feed products.

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[DPA 15]

"*VeGarum*": An innovative, healthy and tasty seaweed "*Garum*"

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The modern food consumption trends are compelling the demand for innovative, sustainable, diverse, healthy and flavourful products, that complement specific market segments[1]. This is leading to approaches focused on food waste reduction, natural and local ingredients and story-telling gastronomicexperiences. Seaweeds are alternative marine resources, highly nutritive and rich in umami flavour compounds, ideal for the production of new vegetable-based products, such as fermented foods, an important trend of consumption associated to ethnic and cultural identity[2].
During the Roman Era, fermented fish products as "Garum", were widely produced in Portugal[3]. This work aims to develop new sauce products with seaweeds from the Portuguese coast ("*VeGarum*"), combining seaweed and fermentation benefits to obtain a flavourful and healthy product.
Two macroalgae were selected, one green (Ulva rigida) and other red (Palmaria palmata), based on their nutritional composition. Macroalgae were fermented in the presence of 5% (w/w) sea salt, using their autochthonous microbiota together with rice koji (rice with Aspergillus oryzae), which provides amylases, proteases and pectinases for the enzymatic hydrolysis. The evolution of pH, soluble solids and sugar consumption and fermentation metabolites was followed.
Glucose increased initially, due to enzymatic activity, being totally depleted by the end of fermentation with production of ethanol and acetic and lactic acid, suggesting the existence of yeast and acetic and lactic acid bacteria in seaweeds microbiota. The products sea aroma improved overtime, becoming milder and more appealing, revealing an umami flavour that may be further improved.
Two high-acidic fermented pastes, safe and well accepted by the consumers, were obtained. These products can be used as sauces to condiment and enhance other foods flavour, or incorporated in products such as salty snacks, pasta and dough. VeGarums can contribute to highlight seaweeds as innovative and healthy alternatives to fish-based fermented products, suitable for vegan and non-veganconsumers.

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[DPA 16]

Process optimization and shelf-life determination of processed food. Review of same case studies.

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Consumers expect processed foods to be safe and have sensory and nutritional quality characteristics similar to fresh products. Food processing has three main objectives: a) to make food safe; b) to provide products with high-quality attributes, transforming them into more convenient or attractive forms for consumption, and c) to extend their shelf life. Food processes, which involve thermal treatments (e.g., blanching, pasteurization, sterilization, or drying) and storage that resort to the use of temperatures such as freezing and refrigeration, are processes that depend on the applied temperature-time binomials [1].
Different mathematical models that describe and/or predict changes in the characteristics of processed foods under constant real or dynamic conditions are fundamental tools in the development of new products, process optimization, and determination of the lifetime of these foods in different scenarios. In this study, the quality of certain vegetables (carrots, pumpkin, broccoli) was modelled as a function of specific temperature profiles to which they were subjected. Optimisation of the blanching operation according to different quality attributes, inactivation of peroxidase activity [2, 3] and determination of their shelf life during frozen storage under isothermal and non-isothermal conditions will be presented and discussed [4, 5].

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[DPA 17]

Consumer's acceptance of beers enriched with elderberry berries (*Sambucus nigra*)

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In this work, the richness and evolution of the sensations in the consumption of two different styles of beer (Blond Ale and Catharina Sour) containing elderberry berries (*Sambucus nigra*), and the temporal comparison of the sensations (descriptive and hedonic) were evaluated. Beers were prepared at an industrial pilot scale. Physicochemical and sensory analyses were carried out, and elderberry anthocyanins were quantified, to assess the quality of the beers and the transference of bioactive compounds. TDS (Temporal Dominance of Sensations) and TDL (Temporal Drivers of Liking) were applied as sensorial evaluation methodologies to consider the dynamic nature of drinking, evaluating the sensory perception and liking score of the beers as they change during the tasting. The list of TDS included eight attributes: sweet, malt, red fruits, bitter, hops, acid, sour and yeast. The duration of dominance was calculated; corresponding to the time elapsed between its selection and the change to another attribute. Ninety regular beer consumers tasted both beers. Statistical analyses were done with R (R version 4.0.2, RStudio team, 2020).
Results highlighted that if a certain attribute was never selected, the duration of its dominance would be null. The difference of TDS curves between both beers allowed a direct comparison and hedonic temporal data of the TDS profiles indicated higher preference values for Blond beer, compared to Sour. Pasteurization did not cause any chemical and sensory damage. The proposed approach is a useful tool for the launching of new beers, as it allows to obtain dominance curves for each attribute over time and to fully characterize the richness and evolution of sensations in the consumption of each type of beer.

[DPA 18]

Influence of modified atmosphere in the shelf-life of packaged chicken liver: study in an industrial environment

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Poultry meat and offals are very perishable foods. To keep these foods fresh and safe, modified atmosphere packaging (MAP) is often used [1]. The atmosphere inside these packages is composed by a mixture of gases suitable for each food, in order to provide freshness and quality for a longer period of time [2-3].
Meeting the interests of the company Lusiaves S.A., where this work was carried out, the aim was to assess the cause of the changes in chicken liver, as well as the changes in the respective packaging, responsible for the reduced shelf-life of these samples. Within 5 days, after packaging the samples, the percentage of gases inside the package was monitored and changes in the color of the livers and in the package were recorded.
Assays were performed with the mixture commonly used in modified atmosphere to pack poultry livers (55-70%O_2:20-30%CO_2:3-5%N_2, briefly designated 70%O_2:30%CO_2). Subsequently, two other mixtures were proposed: 75%O_2:25%CO_2 and 80%O_2:20%CO_2. The new mixtures allowed to delay the appearance of changes both in the color of the livers and in the packaging, which increased the shelf life of packaged poultry livers. In the case of the MAP with 70%O_2:30%CO_2, the color changes started on the third day after packaging, but in the case of the other two mixtures, the color changes only started on the fifth day. Regarding the changes in the packaging, in the case of MAP with 70%O_2:30%CO_2, the changes started on the second day after packaging, while in the case of other gas mixtures, they started on the third day. Possible causes for these results were discussed and additional studies that could increase the shelf life of viscera in modified atmosphere packaging were proposed.

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[DPA 19]

Development of functional pectin edible films prepared using couve galega (Brassica oleracea L.) by-products.

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Brassicaceae are considered important vegetables due to evidence of their health promoting effects associated with bioactive compounds present in the edible parts of the plants [1]. Cabbage processing produces a high quantity of by-products (leaves and stems) with no recovery in the fresh-cut industry. The recovery of this vegetal matter is of interest for the development of new food ingredients, while reducing food waste. The present work studied the development of edible functional composite films based on pectin and using “couve galega” (*Brassica oleracea* L.) powder as fillers obtained from by-products of the fresh-cut industry [2]. The total phenolic content (TPC) and the antioxidant capacity (DPPH, ABTS and FRAP) of the brassica by-products, after blanching, drying and milling to powder, and of the films (with incorporation of different concentrations of powders, 0%, 5%, 10%, 20%) were studied. Furthermore, the correlation between antioxidant activity and phenolic content was evaluated.

According to the results obtained, fresh by-products had a TPC of 39.5 mg EAG/100 g FW. They showed a free radical scavenging (DPPH and ABTS radicals, ferric reducing ability (FRAP assay)) antioxidant capacity of 600 mg EAG/100g FW, 691 µmol/g 2103 mg and EAG/100g PW, respectively. The blanching, drying and milling operation did not affect ($p > 0.05$) the bioactivity of the samples. On the other hand, TPC and antioxidant capacity of films increased linearly with powder added. Also, the film's antioxidant activity is directly related to the concentrations of dehydrated Brassicca powder added. For amounts of powder greater than or equal to 5%, films with similar antioxidant levels were obtained to those evaluated in the raw material Brassica. Another finding that can be emphasized is that the antioxidant activity, particularly with DPPH and FRAP essays, was highly correlated with the phenolic content (0.99 and 0.95, respectively). The results underline the potential of using "couve galega" brassica by-products as an inexpensive source of antioxidant compounds and functional ingredient in the formulation packaging films.

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[DPA 20]

Plant-based beverages fortified with resveratrol complexes of β and γ cyclodextrins: impact on bioaccessibility

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Resveratrol (RSV) is a functional ingredient with numerous bioactive properties, but precautions should be considered in the formulation of liquid food products due its poor solubility and bioavailability. In this context, cyclodextrin complexes are a viable strategy, already applied in delivering hydrophobic compounds [1]. This study aimed at evaluating the use of β and γ-cyclodextrin complexes with RSV (β-CD-RSV and γ CD RSV, respectively) on rice beverages (BA), concerning the impacts on RSV solubility and bioaccessibility.
BA was fortified with 0.75 mg/mL of RSV (free or complexed with CDs) and submitted to digestion in static and dynamic models. Static digestion (SD) was performed according to consensus Infogest 2.2 parameters [2], on one-pot Erlenmeyer at 37ºC. Upon digestion, the BA samples were centrifuged, resulting in sediment (digest, composed of insoluble compounds) and a supernatant (micellar phase). Dynamic digestion (DD) was performed in a dynamic gastrointestinal model, miming gastric, duodenal, jejunal, and ileal phases [3]. The filtered resulting samples in each digestion compartment were extracted with ethyl acetate (1:1), evaporated and resuspended in 70% ethanol. RSV quantification of non-digested and digested BA (with both digestion methodologies) was estimated by UV-Vis spectrometry at 306 nm. The RSV bioaccessibility was calculated by the quotient between RSV in the micellar phase and digest phase (for SD) and by the quotient between the sum of RSV in jejunum and ileum phase by the RSV in the duodenum (for DD).
The results indicated that, even with CD inclusion, the solubility of RSV in BA was not significantly changed. Cyclodextrin inclusion afforded an increase of RSV bioaccessibility upon digestion (about 24 and 27% in SD and DD, respectively), particularly for γ CD RSV. According to DD results, inclusion of RSV into CDs could be the reason for the increase in RSV bioaccessibility, mainly in the filtered intestinal phases.

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[DPA 21]

Stabilization of raspberry fruits followed by application in muffins

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Raspberry is characterized by its beneficial effects inherent to its anthocyanin profile [1]. Due to its high perishability, 1/3 of its production is wasted, making its valuation essential. In this work, freeze-drying and convective drying at 30 and 40 ºC were used for fruits stabilization and subsequent incorporation into muffin formulations. The two red raspberry varieties under study, *Pacific Deluxe* and *Versailles* had similar colour (*CIELab* parameters) and similar profile and content in phenolic compounds. When evaluating the impact of dehydration techniques on the two varieties, freeze drying was the one that did not show significant differences in terms of structure, colour, composition of phenolic compounds and antioxidant activity, when compared to fresh raspberry fruits. On the other hand, dried raspberries through both convective drying conditions showed significant differences in these parameters, with a significant reduction in the content of phenolic compounds as well as on their antioxidant activity. Besides, no significant differences were observed among the composition of 2 varieties and drying at 30 ºC revealed a higher impact in the colour of the dried fruits.
The incorporation of *Versailles* raspberry fruits in fresh, freeze-dried, and dried at 40 °C forms into muffin formulations resulted in products with different colours: fresh raspberries showed a green colour, which was attenuated in muffins made with dried raspberries and non-existent in muffins with freeze-dried raspberries. This greenish colour, resulting from the impact of alkaline pH of the dough, was enhanced by the syneresis phenomenon when using fresh raspberries. Sensory analysis revealed good acceptance of all muffin formulations, however, those containing freeze-dried samples proved to be the most appreciated in terms of fruit appearance and sweet/acid balance. Thus, showing a promising approach for developing a new product, promoting the valorisation and sale of raspberry fruit waste under a circular economy concept.

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Dare 2 Change

Food & Consumer Trends

[TAC 1]

Beef Consumption in a balanced diet

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Beef is an excellent source of high-quality protein, B-vitamins and trace minerals, having an important role in food and nutrition security [1].
In the last decade red meat consumption has been discouraged due to the reported relationship between meat consumption and diseases like diabetes, obesity, atherosclerosis, coronary heart diseaseand some cancer types [2]. Recently, several studies have shown that these relationship between beefconsumption and those diseases may be due to other factors, which were not considered in the studiesperformed (smoking, sedentary lifestyle/lack of physical exercise, among others), and not to the actualmeat consumption [3].
The quantity and composition of beef fat has also been pointed as reasons to decrease its' consumption [1]. However, regarding the amount of beef fat, the differences between production and consumption habits between countries are large. In Portugal, the fat content of beef generally varies between 1 and 3 %, being considered a very lean meat [4]. Regarding the lipid profile, beef is rich in saturated fatty acids, however, contrary to what was advocated in the past, it is now known that some of these fatty acids do not have a negative effect on human health, being instead neutral, such as those existing in greater quantity in beef [5].
It has been reported that diets, in which meat consumption has been strictly restricted, are causing somehealth problems, namely anemia, due to low iron intake [6]. If food excesses are an important nutritionalproblem, excessively restrictive diets may be the origin of a malnutrition problem with important consequences for human health, being important to look at them with the same attention. Therefore, current dietary recommendations consider beef has an essential part of a balanced diet not posing a problem for human health [2,3,6], contrary to what is defended in many forums.

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[TAC 2]

Market trends in mood and energy-boosting food

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The pandemic caused by the SARS-CoV-2 virus has considerably accelerated the change in food consumption habits, resulting in fast adaptation of the agri-food sector[1]. Now more than ever, consumers are concerned about their lifestyle and focus on improving mental/physical health and strengthening their immune system. We intend to describe the substances with the highest prevalence and with claims "relaxation and mood" and "energy power" in food and beverages (F&B) launched in the European market, providing an overview of regulatory status and their main mechanisms of action. Products containing soothing substances like L-tyrosine, L-theanine, cannabidiol, melatonin, excluding food supplements, increased 22% annually from 2016 to 2020[2]. Products in nutritional, sports & energy drinks, and baby food categories, along with functional claims of energy, brain and nervous system and/or vitamins and minerals fortification were the majority of new launches. According to Reg. (EU) No 432/2012, the health claim of "contribution to normal energy-yielding metabolism" may be used only for food that is at least "a source of" calcium, copper, iodine, iron, magnesium, manganese, phosphorus, thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamin B6, B12 and/or C. Iron is the ingredient most used[2] among the potential ergogenic aids (substances or devices that athletes use to increase energy, performance and recovery)[3], it is mainly added in bakery, meals, breakfast cereals and baby food, and these products represent 3.4% of total new launches from 2016 to October 2021. Vitamin B12 sources have a global penetration of 1.8%, especially in dairy and sports & energy drinks. Nutritional and energy drinks categories represent 7% of new launches with beetroot added, but represent almost 50% for caffeine application in F&B. Creatine, citrulline malate and coenzyme Q10 are used in nutritional drinks, though having minor penetration in the F&B categories, but relevant as supplements.

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[TAC 3]

Use of temporal check-all-that-apply with emotions (TCATA-E) on the dynamic emotional perception of ice creams

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Measuring food-evoked emotions can help to understand food choices and consumer's behaviour and, therefore, developing new products. Furthermore, eating context can influence consumer's response to food. The dynamic method Temporal Check-All-That-Apply (TCATA) allows to access the evolution of different attributes simultaneously throughout consumption, and it is usually applied with sensations [1-3]. This study aimed to develop a procedure to apply the TCATA method with emotions (TCATA-E), and to evaluate the impact of thermal sensation on the emotional dynamic profile of five vanilla ice creams (two regular dairy ice creams- one national and one international, one vegan ice cream, one dairy ice cream with no-added sugar, and one dairy organic ice cream).
A systematic review based on the use of emotions on ice cream evaluation, was performed to collect emotion terms related with the product category. Then, six focus group were carried out aiming to create a shorter list, comprising twelve emotions for the TCATA-E evaluation. A panel of eighty consumers evaluated five samples tested at three different ambient temperatures: 16, 21 and 26 °C. Consumers were told to take only one bite of each product. The TCATA-E procedure was performed for 30 seconds, and attribute fading was set to 8 seconds.
Results showed that the national regular ice cream evoked happier when consumed at 26 ºC, comparing to 16 ºC, while dairy ice cream with no-added sugar evoke lower proportion of happy emotion when consumed at 26 ºC. The dairy organic ice cream obtained a significant lower proportion of citations for excited when consumed at 26 ºC. Regarding the international regular ice cream, consumers felt significantly more satisfied when consumed at 16 and 26 ºC, rather than at 21 ºC.
TCATA-E proved to be a good method on the determination of the ice cream emotional dynamic profile, and on the evaluation of the impact of ambient temperature.

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[TAC 4]

Sensory analysis performed within an immersive mixed reality system: impact on engagement and overall liking

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Traditionally, sensory analysis methodologies are performed on sensory booths which are designed to minimize external stimuli. Nonetheless, evaluations performed in these traditional conditions lack ecological validity (balance between scientific control and the components of natural environment) and do not always predict the success of the products in the market. Virtual reality may lead to a high level of presence but faces several restrictions when evaluating real products as it is hard for the participant to interact with them [1]. The main goal of this study was to create an immersive mixed reality system, where a real product and the participant are placed into a virtual environment, and to evaluate the impact of this on the product overall liking score.
After developing the mixed reality system, a panel of 102 young adult consumers (aged 18-45 years) evaluated five different samples of commercial peach nectars. Evaluation occurred during three sessions, in three different environments: dining room, school cafeteria (both in virtual environments) and laboratory (real life sensory booth), following a balanced design with a one-week interval between sessions. Consumers rated overall liking on a 9-point hedonic scale, followed by open comments. At each session, participants tasted the five samples following a balanced sequential monadic presentation. After each session, consumers answered a 10-item Engagement Questionnaire [2] and a 6-item Presence Questionnaire [3], specific for virtual environments.
The type of environment affected the hedonic discrimination between samples, with the immersive mixed reality environments promoting a higher discrimination between samples than in the laboratory setting. On the other hand, the level of engagement was significantly higher when using the mixed reality system, with this effect being more evident in the cafeteria and for the affective value sub-scale. Similarly, the level of presence in the virtual environment, was significantly higher in the cafeteria environment.

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[TAC 5]

Evaluation of the impact of different fading times on the sensory dynamic characterization of *Queijo São Jorge* (PDO) cheese and overall liking

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Temporal methods such as the Temporal Check-All-That-Apply (TCATA), are used to obtain dynamic sensory characterization of products. The application of attribute selection fading on TCATA emerged to overcome a difficulty perceived on the TCATA performance [1-2]. It consists on the automatic and gradually deselection of selected attributes. The present work aimed to compare two different fading times (4 and 8 seconds) with the TCATA without fading, evaluating the impact on overall liking and on sensory characterization on a Protected Designation of Origin (PDO) cheese.

A panel of 60 consumers evaluated three *Queijo São Jorge* (PDO) cheese samples with 4, 7 and 12-months of maturing. Evaluation occurred during three sessions, applying three different temporal conditions: TCATA, TCATA-4s and TCATA-8s, following a balanced design with a one-day interval between sessions. Consumers were asked to evaluate the samples with a previously designed ballot comprising twelve attributes, over 120 seconds per sample, following a balanced order of presentation. After each temporal evaluation, consumers rated overall liking on a 9-point hedonic scale.

Results showed that TCATA-4s presented the lowest citation proportion for all studies. Differences were found between TCATA and TCATA-8s in the attribute selection. However, TCATA-8s revealed a lower citation proportion but revealed more discriminative results. Moreover, the 4-months sample was described as smooth, salty and soft, the 7-months was described as spicy, strong and salty, and the 12-months was described as strong, salty, bitter and spicy, for all TCATA evaluations (with and without fading). Samples with 4- and 7-months maturing tended to present higher liking scores with significant differences in TCATA without fading.

The present study suggests that fading contributes to provide good quality data for creation of the dynamic sensory profile of *Queijo São Jorge* (PDO) cheese with different maturing times, as well as for discrimination between samples.

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[TAC 6]

Using Emoji to evaluate consumers' perception of foods

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The use of emoji for food- related emotion research has recently gained attention, as they are familiar to consumers and can be used in cross-cultural research [1-3]. This study aimed to apply emojis as a tool for measuring consumers' emotional perception of food products.
A preliminary list of emojis was developed, based on a systematic review. Tests were conducted with 200 participants divided into age groups (6-12, 13-17, 18-30, 31-40 y.o.). Participants were instructed to indicate the emotion or feeling that 51 emoji convey. Complementarily, a card sorting task, where participants were given 51 printed cards with emoji and asked to group them according to similarities, considering a food context. Each sorted group was defined with an emotion word. The results obtained from the previous task were used to develop a reduced set of emoji to be applied in an evoked emotions tests during tasting. 50 participants from each age groups evaluated six juices in an emoji CATA ballot, after overall liking evaluation. In a different session, participants were instructed to repeat the evaluation, while exposed to the juice's package.
For the semantic categorisation, strong differences were found across emoji. The responses related to emoji's valence were similar regardless of age, while arousal distinction was not well-marked for positive emojis. Using the data from sorting analysis complemented with the semantic results, it was possible to design a reduced list of emojis, which was useful to distinguish samples that were not discriminated based on their mean liking scores. When introducing extrinsic factors, the brand/package had a strong influence increasing mean liking scores.
This research brought new aspects to the emojis utilization when evaluating their conceptualization segmented by age groups in food context, proposing a consumer-oriented emoji list which reveal as a valuable alternative on the emotional response evaluation.

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[TAC 7]

Agri-food National Brand: A New Perspective on Portuguese Gastronomy

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This research highlights the evolution of agri-food and gastronomy history from the Middle Ages to the present day. It is a chance to recover the historical legacy, in culture, tradition, pleasure and happiness at the table, defining a national brand to protect and promote the uniqueness of Portuguese gastronomy.
The challenge of this research is to explore this identity based on a past concept, Petisco, which can be transformed into an emerging one.
Petisco has been defined as a culturally Portuguese consumption practice and food conviviality. Based on the evolution of a Portuguese gastronomic identity, built and adapted over the past 340 years (1680 - 2020). Represents the celebrations that brought people together around the table, regardless age and social class. It is a timeless and inclusive concept that is part of history and that by carrying over to the present day, will symbolize the legacy and modernity of Portuguese enogastronomy.
One way to address the uncertainties and explore the potential actions for the applicability of the Petisco concept was the use forecasting techniques.
In this research the Delphi method was applied, a technique that enables the development of two rounds of ten-year long-term forecasting (2021 - 2031). As well as an auscultation to measure the degree of self-knowledge of the experts. Nineteen national experts from the academic, public and private sectors contributed, in a set of thirty-six statements applied in the two rounds.
After analyzing the statements, 3 had a low degree of consensus, 26 had a medium degree of consensus and 7 stand out with a high degree of consensus.

Keywords: Petisco, Portuguese Gastronomy, Brand, Delphi and Trends.

Dare 2 Change

Health & Wellbeing

[SBE 1]

Enterotoxin encoding genes and antibiotic resistance found in coagulase positive and coagulase negative *Staphylococcus* isolatesfrom RTE street food sold in Maputo, Mozambique

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These SEs have often been associated with coagulase positive staphylococci (CPS). However, reports are beginning to emerge of the association of food poisoning enterotoxins with coagulase negative staphylococci (CNS). In addition to SEs, the increase in methicillin-resistant Staphylococcus (MRSA) strains another problem associated with staphylococci. The aim of this study was to evaluate the presence of staphylococcal enterotoxin genes and antibiotic resistance in 70 Staphylococcus spp. (51 CNS and 19 CPS) recovered from ready-to-eat (RTE) street food sold in Maputo, Mozambique. The Multiplex PCR (MPCR) technique was used to search for six virulent genes, five for SEs (sea, seb, sec, sed, see) and hlb. The same MPCR approach was used to search for seven genes encoding penicillin, methicillin, and erythromycin resistance (BlaZ, mecA, vancA, vancB, ermA, ermB and ermC). In SCP isolates, 57.9% showed genes encoding for at least one of the six toxins accessed in this study. The hlb gene was the most frequent (62.5%), followed by the sec and sed genes (12.5%), respectively. In SCN isolates, 51% showed genes that code for at least one of the six virulent factors. The most frequent gene was sec with a prevalence of 48.6%, followed by hlb with 22.9% and sed with 17.1%. The BlaZ gene was the most frequent both in the CPS (42.1%) and in the CNS (82.4%), followed by the mecA and vancA gene which represented 36.8% and 31.6% in the SCP isolates, and 43.1% in the SCN isolates, respectively. These results show the need to give CNS isolates the same research importance as CPS isolates, as both can be potentially dangerous when it comes to the possibility of causing food poisoning, given the presence of genes encoding SEs. In addition, the CNS showed the potential to spread antibiotic resistance genes.

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[SBE 2]

Aptamers For Detection of Staphylococcal Enterotoxins in Food Samples

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Staphylococcal food poisoning is a gastrointestinal disease caused by the consumption of food containing toxins pre-formed by enterotoxigenic Staphylococcus. Current detection of these toxins relies on time-consuming antibody-based immunoassays associated with cross-reactivity, low sensitivity, and interference from food matrices [1]. Aptamers are single-stranded oligonucleotides with a defined three- dimensional shape that bind with high-affinity to a target molecule. They are selected by the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) method which consists in the screening of a random oligonucleotide library down to highly specific sequences for the target, by the repetition of successive steps of selection, amplification, and conditioning. Applying unnatural nucleotides is an essential evolution of aptamers, considering the limitations in the chemical and biological stability of traditional DNA and RNA aptamers [2].

In this work, a SELEX procedure was applied to isolate aptamers with unnatural nucleotides (2'-deoxy- 2'-fluoroarabinonucleotides (FANA)) specific to staphylococcal enterotoxin A (SEA). After performing 12 rounds of selection, 24 potential FANA aptamers for SEA were identified. Although the nucleotide comparison shows no consensus sequences between selected oligonucleotides, the analysis of secondary structures reveals enrichment in similarly folding patterns. These results reinforce the potential of applying unnatural nucleotides in SELEX procedures and suggest that, for SEA, aptamer selection points to preferential secondary structures which are like other DNA aptamers already described [3].

This work was the start point to create a platform for the development of nucleic acid mimics aptamers that combines aptamers biorecognition ability and unnatural nucleotides diversity/increased affinity. The selected aptamers will then be incorporated into a portable assay that can be easily implemented at the food industry; and tested in real food matrices for validation and comparison with standard detection systems. Although it has been developed for SEA, this technological platform might be easily adapted to any food poisoning toxin or protein.

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[SBE 3]

Effect of phytic acid on the viscosities and starch hydrolysis of rice varieties

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The phytic acid (PA) is an organic acid with beneficial antioxidant and hypolipidemic effects[1], due to its power to suppress oxidative reactions. PA can be found mainly in seeds and cereal bran, particularly in rice bran its content can vary from 4 to 22 g/100 g[2,3]. PA was also described with potential effects in controlling diabetes[4]. These benefits for diabetes are related to the prevention of hyperglycemia by reducing rice glycemic index (GI) through inhibition of the salivary and pancreatic amylase enzyme. GI can be estimated by '*in vitro*' starch hydrolysis which can be related with the rice viscosities profiles[5].
This study aimed to identify and understand the effect of PA on starch digestibility. A mechanism of inhibition of the alpha-amylase enzyme was tested through the assessment of the effect of PA on the viscosity parameters in the presence of the enzyme. Simultaneously, the relationship between PA concentrations, viscosity profiles and glycemic response were investigated in different rice samples ('Basmati', 'Ceres', 'Maçarico').
The PA concentrations are negative correlated with peak viscosities ($r= -0.5$, $p<0.05$). Ceres and Basmati varieties with the highest initial viscosity peak showed a greater loss of viscosity after enzyme action, unlike the Maçarico variety. When PA is added to all rice flour varieties there is a recovery of the viscosity profile, which confirms the inhibitory effect of PA on the alpha-amylase activity. As for the estimated glycemic index (GI), it was verified that the Maçarico was the variety with the lowest GI (83.71) and the highest PA content (12.2 g/100 g of bran). Although phytic acid is often described as an antinutrient, these results show that PA could be an important potential bioactive for delaying starch hydrolysis with impact in the reduction of rice glycemic index.

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[SBE 4]

Multi-screening of veterinary medicines in milk by UHPLC-ToF-MS

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The use of veterinary medicines in animal production is a common practice to guarantee animal welfare and to prevent economical losses due to animal health problems. The arising of consumer's awareness concerning the health risks associated with the presence of residues of veterinary drugs in food products, the origin and quality control of food, brings Food Safety to a prominent position in terms of public health. For European Commission, Food Safety is one of the top priorities and for that reason, analytical tools to provide the means to monitor the presence of medicines in products of animal origin needs constant update and improvements. The analysis of compounds belonging to diverse groups of compounds, in complex biological samples, is often time-consuming in terms of sample preparation, usually requiring different extraction procedures and different detection approaches.

The present method describes a multi-screening for residues of veterinary drugs resorting to ultra-high performance liquid chromatography coupled with a time-of-flight mass spectrometer (UHPLC-ToF-MS) technology. The aim is to monitor a high number of compounds, from different classes of drugs, in milk of cow, goat and sheep, using a fast and generic sample preparation and a high-resolution mass spectrometry (HR-MS) detection, in a single chromatographic run. Medicines belonging to nitroimidazoles, coccidiostats, antibiotics, anthelmintics, NSAIDs, corticosteroids and sedatives can be detected at the screening target concentration (STC) that is related with the maximum residue limit (MRL) or recommended concentration from the European Reference Laboratories [1,2]. The STC is the limit above which a sample is considered "potential non-compliant" and a confirmatory test is triggered.

Despite the high cost associated with HR-MS, it must be highlighted that the full-scan data acquisition obtained with those detectors allows a retrospective analysis for other additional compounds, not currently targeted [3].

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Development and application of analytical methodologies for determination of Amanitins in urine, liver and bile samples, by UHPLC-MS/MS

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Although the consumption of the majority of the existing mushrooms is harmless, there are some species constituted by toxic substances, whose consumption causes the development of noxious effects in different organs and tissues. Amanitins are highly toxic cyclopeptides, isolated from various Amanita mushroom species, considered the most potent poisons accounting for the toxic effects, and may be considered the deadliest of all accidental ingestions.
Despite the attempts to define different forms of distinguish the edible mushrooms from the toxic ones, none of them is secure enough and some people still erroneously believe that they can follow feasible rules to guarantee the distinction. In fact, the non-acquaintance and the false quoted convictions, more than the eventual confusions, represents the main cause for mushroom poisoning, a common issue especially in forest regions where wild mushrooms grows and the harvesting habit and consumption is very common.
In order to conclude about a potential case of poisoning and follow the necessary emergency procedures, is necessary to have rapid/accurate/sensitive methods to detect the presence of those toxins in biological samples and proceed as needed [1]. The present study was focused on the development and validation of analytical methodologies by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for the detection of α- and β-amanitin in urine, liver and bile. The developed method was validated through the evaluation of linearity, specificity, robustness, recovery, and precision, limit of detection (LOD) and limit of quantification (LOQ). Calculated LODs, LOQs range from 0.2 to 10.9 $\mu g.kg^{-1}$, and 0.6 to 14.7 $\mu g.kg^{-1}$ for α-amanitin, 0.2 to 9.7 $\mu g.kg^{-1}$, and 0.5 to 12.3 $\mu g.kg^{-1}$ for β-amanitin. In terms of precision, both compounds follow below 13% and recovery over 90%. This represents a high and unique analytical throughput in amanitin poisoning that encompasses several target matrices, allowing to efficiently responding to this fatal problem.

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[SBE 6]

Metagenomic assessment of the microbial diversity of swine carcasses

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Contamination, cross-contamination, or carcass recontamination can mainly occur along the slaughtering processes due to contact between animals during their transportation, steps of slaughter carcass preparation, slaughterhouse environment, clothing, and poor slaughter and hygiene practices [1]. Evaluating the microbiological profile is important to verify hygiene conditions during the slaughter processes and ensure the food safety of the final product [2]. The purpose of this study was to characterize the microbial community of swine carcasses and environmental samples - knives and drains - using a metagenomic approach. Gauze swabs were used to collect samples from drains and from five animals before and after evisceration, and water from the knives sterilizer was collected in sterile tubes before and after eviscerations. Samples were prepared for Illumina Sequencing by 16S rRNA gene amplification of the bacterial community. All 16S rRNA data were analysed with Kraken v1 using the pre-built MiniKraken 8Gb database (https://ccb.jhu.edu/software/kraken/) with default parameters. Twenty-five genera with abundance above 1% were found. The most abundant genus on carcasses was *Anoxybacillus* (52% of the total microbiome) and on environmental samples *Oenococcus* (15%). *Enterobacteriaceae* were detected in 13 collected points (prevalence ranging from 0.001% to 0.02%), with different genera found: *Citrobacter* (18.0%), *Klebsiella* (14.0%), *Erwinia* (12.1%), *Enterobacter* (9.99%), *Trabulsiella* (5.99%), *Providencia* (4.0%), *Serratia* (4.0%), *Salmonella* (4.0%) and *Morganella* (1.99%). It is important to highlight that *Salmonella* spp. was found in only two samples, one from the carcass and the other from the drain sampled on the same day. Other genera of interest in food microbiology were also detected, namely, *Pseudomonas*, *Lactobacillus*, *Campylobacter*, *Clostridium*, *Bacillus*, *Staphylococcus*, and *Lactococcus*. Such diversity suggests possible interventions to extend shelf life hampered by the microbial consortia with pork products. Otherwise, it may be useful for developing microbial traceability to a slaughterhouse.

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[SBE 7]

Confirmation of Carbadox and Olaquindox residues in muscle and liver by UHPLC-MS/MS

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Carbadox and olaquindox drugs derived from the quinoxalines, a group of synthetic antibacterial veterinary drugs that have bicyclic heteroaromatic systems as the basic structure. The carbadox (CBX) and olaquindox (OLQ) were widely used in poultry and swine diets to improve feed efficiency, to increase the rate of weight gain and assisted in the prevention of bacterial enteritis. Both carbadox and olaquindox were approved as feed additives in 1974 and 1976, respectively. However, in 1998 the European Commission (EC) banned the use of CBX and OLQ in food animal production regarding possible carcinogenic, mutagenic and photoallergenic effects of the drugs [1]. After administration, the CBX and OLQ are quickly metabolized, by a deoxygenation reaction, to more stable products: carbadox to quinoxaline-2-carboxylic (QCA) and olaquindox to 3-methylquinoxaline-2-carboxylic acid (MQCA). Because they remain in tissue longer that the parent compound, QCA and MQCA were designed as marker residues. It is also recommended that the analytical methods used to detect and quantify those compounds should guarantee minimum method performance requirements (MMPRs) below 5 µg/Kg in muscle and liver [2].
To monitor the presence of those metabolites, a simple sample treatment consisting of acid deproteination followed by a liquid-liquid extraction step, was developed. Analysis was performed using a C18 column coupled to electrospray MS/MS, operated in positive mode, with detection by UHPLC-MS/MS. This methodology was fully validated in accordance with the requirements for confirmatory criteria, described in the European Commission Decision 2002/657/EC [3].
The developed method will be applied in routine analysis of swine meat and liver samples in order to control the abusive use of the referred quinoxalines.

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[SBE 8]

Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw milk from Northern Portugal

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Staphylococcus aureus and its enterotoxins (SEs) are a serious and costly concern for milk industry and public health [1]. This work aimed to characterize the prevalence of *S. aureus* and enterotoxins in raw milk collected in the main dairy basin region of Portugal mainland. The presence of *S. aureus* was confirmed in 53% of refrigerated raw milk samples collected from the bulk tank of 100 dairy farms according to the ISO standards. Nonetheless, *S. aureus* was always below 10^6 CFU/mL, the minimal concentration expected for enterotoxin production [2]. The presence of enterotoxins- (*sea, seb, sec, sed, see, seg, seh, sei, sej, sep, ser*) and methicillin resistance-encoding genes (*mecA* and *mecC*) was evaluated by PCR. Five isolates were found to be methicillin-resistant *Staphylococcus aureus* (MRSA) and 29 isolates contained enterotoxin-encoding genes. One isolate was positive for *sea*, 3 isolates were positive for *seh*, 4 isolates were positive for *sec*, 25 isolates were positive for *sei* and 26 isolates were positive for *seg*. Consistent with other reports, *seg and sei* coexisted in most isolates, *seg* was only detected independently of the other gene in one isolate [3, 4]. *Sec* was only found in conjunction with *seg* and *sei*. *Seh* was detected alone or together with *sea*. Overall, the occurrence of non-typical enterotoxin genes (*seg*, *seh* and *sei*) was higher than the "top five" genes (*sea-see*). The detection of SEs (SEA-SEE), according to the EU-RL standard, revealed one positive sample. Interestingly, *S. aureus* was not detected on the positive sample, demonstrating that SEs can be present without requiring the presence of the bacteria [5]. These results suggest that raw milk can be an important source of MRSA, enterotoxigenic *S. aureus* and enterotoxins. Surveillance and postharvest handling practices might be crucial to prevent the spread along the food chain.

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[SBE 9]

Optimization of nanoemulsions based on plant-derived proteins for food applications

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Recently, there has been a growing interest in plant-derived proteins due to their interesting properties and the trend to replace animal-derived proteins [1]. One of the emerging interests is their use to develop nanoemulsions that are kinetically stabilized by their small dimension, unlike classic emulsions [2].
In this work, oil-in-water nanoemulsions were produced using potato (Solanum tuberosum) or lupin (Lupinus angustifolius) protein as emulsifiers and high-speed homogenization, followed by ultrasonic homogenization (US). A central composite rotatable experimental design was used to evaluate the influence of three independent variables: water/oil ratio (65-75% of water), protein content (1-6%) and US time (1-7 min) on the size average (by intensity) and polydispersity index (PdI) of the nanoemulsions. A total of 17 experiments were performed with 14 three-level experimental points, and 3 replicates at the central point. The best conditions for each protein were selected for stability studies during storage at 4 ºC or 20 ºC. Freshly made nanoemulsions were characterized during storage by size, PdI, zeta- potential and visual observation. The smallest mean droplet size for potato protein was 439.9 nm and PdI value 0.464 [21:73 (w/w) oil/water ratio, 6% of protein and 6 min of US]. The smallest mean droplet size for lupin protein was 505.5 nm and PdI value 0.434 [23.6:73 (w/w) oil/water ratio, 3.4% of protein and 6 min of US]. The nanoemulsions with potato protein remained stable (size, zeta-potential and visually) when stored in 4 ºC and 20 ºC for 24 days, without phase separation. Lupin based nanoemulsions stored at 4 ºC remained without phase separation for 18 days, while those stored at 20 ºC showed a phase separation after 4 days.
Results showed that plant-derived proteins can be used as emulsifiers and in combination with ultrasonic homogenization have the potential to produce stable nanoemulsions, foreseeing different applications in the food industry.

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[SBE 10]

Effect of onion flavonoids in bioaccesibility, bioavailability and further immunogenicity of peanut proteins

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Peanut allergy (PA)[1] is one of the primary forms of allergy and may induce severe and life-threatening allergic reactions. The mainstay of treatment is the elimination of peanut proteins from the diet[2]. Growing research have been related the effect of dietary patterns in food allergy[3, 4]. The enzymatic modification of proteins during human digestion are closely related with their immunogenicity but the digestion process may be influenced by the way food components interact with each other and with digestive enzymes and ultimately affect human health. Under this context, this study is focused in analysing the effect of flavonoids in peanut proteins bioaccesibility, bioavailability and immunogenicity.
Thus, an onion flavonoid-rich extract was obtained and further fractionated into five fractions with different composition. In parallel, peanut proteins was extracted with 50mM Tris-HCl and characterized by SDS-PAGE. The peanut protein extract were submitted to in vitro human digestion simultaneously with the flavonoids fractions following the INFOGEST protocol[5]. The impact in digestibility was then assayed by characterizing the intact proteins by SDS-PAGE. Likewise, immunogenic peptides from the main allergens released during digestion (Arah1, Arah2, Arah3 and Arah6) were quantified by HRLC-MS/MS in a SRM multiplex acquisition mode. Furthermore, an epithelial transport assay was performed in a Caco-2 cell model tracking the bioavailability of the peanut immunogenic peptides by HRLC-MS/MS.
The isolated onion flavonoids promoted a significant decrease of bioaccesible-bioavailable immunogenic peptides. The effect of peptide bioaccesibility was affected by the flavonols methylation and glycosylation degree. Likewise, Arah1 peptides showed the lower digestibility and the higher bioavailability even in the presence of onion flavonoids. Overall, onion flavonoids clearly influences peanut digestion, absorption and immunogenicity, thus suggesting that the consumption of dietary flavonoids can significantly affect the degree of PA downstream immune reactions.

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[SBE 11]

Measurement of pesticides in fresh legume vegetables using high-performance liquid chromatography coupled to tandem mass spectrometry: a validation study

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Fresh legume vegetables such as beans are a great and not so expensive way to guarantee the right intake of fiber and plant-based protein. They are a group of vegetables quite simple to prepare combined with great health benefits. Green beans and broad beans are largely consumed around the globe, and they are both very rich in different vitamins and minerals, such as vitamin C and magnesium.

To control pests that are prejudicial to different kinds of crops, including crops from this group of vegetables, pesticides are used during their growing. To control their use, and to ensure the food safety, Regulation (EC) No 396/2005 and its amendments establishes maximum residue levels of pesticides in foods [1].

This study aimed to validate a method for monitoring pesticides residues in fresh legume vegetables, using broad beans and green beans as two typical representative vegetables within the category.

The method used for the extraction was a modified QuEChERs method. The procedure used acetonitrile as extraction solvent and a mixture of buffering salts to carry out a liquid–liquid partitioning step. After that, a cleanup step called dispersive solid-phase extraction was performed with primary secondary amine sorbent plus anhydrous $MgSO_4$. The extract was analysed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

A total of 129 pesticides were validated according to the guidance document SANTE/12682/2019 [2]. The limit of quantification was 5 mg/kg for all pesticides except for tolclofos-methyl (10 mg/kg). The method was linear in the range 5-50, 5-60, or 5-70 mg/kg for most of the evaluated pesticides. The method was proved to be precise (2.1-14.4%) and accurate (recovery between 76-106%) at the three spiking levels tested (5,10 and 50 mg/kg).

The method showed to be suitable for monitoring a large number of pesticides residues in fresh legume vegetables.

Acknowledgments: We'd like to thank to our beloved colleague Maria Graça Melo (retired) for the extraordinary work in the development of analytical methods for pesticides residues in fruits and vegetables.

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[SBE 12]

Development and validation of a method for the analysis of 110 pesticides residues in oranges by using modified QuEChERS and HPLC-MS/MS

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Oranges (*Citrus sinensis* L.) are a type of citrus fruit very popular around the globe and largely consumed. According to the Food and Agriculture Organization (FAO), in the year 2019, it was produced in the European union 6097 million tonnes of oranges [1]. This popular citrus fruit is known for the high content in vitamin C, however it is also rich in many other antioxidant compounds that contribute to reduce inflammation which display a protective role against many diseases, such as cancer.

With the growing use of pesticides in agriculture, there is a growing need to achieve methods of determination for this type of residues present in food. The present study was developed in order to validate a method characterized as simple, reliable and economic in orange, as a representative fruit for the citrus group, where lemons, mandarins and tangerines are also included.

To determine the pesticides residues in orange, a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used, followed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). In accordance with de guidance document SANTE/12682/2019 [2], 110 pesticides were validated.

The limit of quantification was 5 mg/kg for all pesticides except for metribuzin (10 mg/kg). 5-50 and 5-60 mg/kg was the range where the method was linear for most of pesticides, except for chlorfenvinphos, ethion, metribuzin, and pirimiphos-methyl. The method proven to be linear, sensitive, precise, and accurate for the 3 spiking levels analyzed (5,10 and 50 mg/kg), where the RSD was between 3.1%-16% and the recovery between 75%-101%.

The analytical methodology showed to be appropriate for monitoring the conformity of citrus fruits with the maximum residue levels for pesticides established by Regulation (EC) No 396/2005 and its amendments [3].

Acknowledgments: We'd like to thank to our beloved colleague Maria Graça Melo (retired) for the extraordinary work in the development of analytical methods for pesticides residues in fruits and vegetables.

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[SBE 13]

Validation of a HPLC-MS/MS method for analysis of 134 pesticides residues in apples

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One of the most appreciated fruits around the globe is apples (*Malus domestica* L.). Apples are a fantastic source of fiber, vitamin C, and potassium. Their antioxidant properties are associated with many health benefits, such as promoting cardiovascular health and prevention of cancer.
In the last century pesticides have become a crucial component in agriculture systems, allowing a visible growth in food production, being apples no exception. Regulation (EC) No 396/2005 was implemented to control their use and establish limits for pesticides residues in food and feed [1].
The present study was designed to validate a reliable, simple, and economic method, for determining pesticides residues in apples, representative of other pome fruits, such as pears.
First, an extraction was performed using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. The extract was analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) with a triple quadrupole instrument using electrospray ionization (ESI). According to the guidance document SANTE/12682/2019 [2], 134 pesticides were validated in apples. Despite paclobutrazol, methacrifos, and fipronil, which limit of quantification (LOQ) was 10 mg/kg, the LOQ for all other pesticides was 5 mg/kg. Depending on the pesticide there were three major ranges where the method was proven to be linear, 5-50 or 5-60 or 5-70 mg/L, except for chlofentezine, coumaphos, ethoprophos, fenamiphos sulfoxide, fipronil, methacrifos, and paclobutrazol. The precision was between 3.7-12.7%, proving to be a precise method. Moreover, the method showed to be accurate because recovery was between 76%-102% at the 3 spiking levels tested (5, 10, and 50 mg/kg).
The methodology has been proven to be efficient, robust, and suitable for monitoring pesticides residues, from different classes, in apples, a representative commodity of the group pome fruits.

Acknowledgments: We'd like to thank to our beloved colleague Maria Graça Melo (retired) for the extraordinary work in the development of analytical methods for pesticides residues in fruits and vegetables.

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[1] EU (2005). Regulation (EC) No 396/2005 of the European Parliament and the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Official Journal of the European Union L70/1-16.
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[SBE 14]

Validation of a Fast Multiresidue Method for Determination of Pesticides in Alliums using Modified QuEChERS and HPLC-MS/MS

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Onions (*Allium cepa* L.) are an important crop worldwide. They vary in shape, size, and colour, being the red, yellow, and white the most common ones. They are a highly valued vegetable since they are very rich in volatile compounds and nutrients, namely vitamin C. Onions are also rich in antioxidant compounds, which can play a protective role against cancer and other diseases.

Pesticides are chemical compounds used to control and combat pests in agricultural areas. Alongside their growing use in this field, there is a need for analytical methodologies that are simultaneously economic, simple, and reliable, in order to monitor pesticides residues in different food samples and assure food safety.

An analytical method was designed and validated to determine pesticides residues in onions, as a representative vegetable of the alliums group, following the guidance document SANTE/12682/2019 [1].

The extraction procedure used modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe method) and the chromatographic analysis was carried out by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). In this study 119 pesticides were validated according to SANTE/12682/2019 [1] guidelines. The limit of quantification (LOQ) for this range of pesticides was 5 mg/kg for all except molecules, except six pesticides which presented a LOQ of 10 mg/kg. The method was proven to be linear in the selected calibration range, sensitive, accurate and precise. Recovery was between 73%-112% and the precision was between 3%-12.9%.

Upon these results, the methodology was found to be efficient and robust, showing to be suitable for monitoring the conformity of samples from the alliums group, such as onions and leeks, concerning a large number of pesticides and taking into account the maximum residue levels in foods established at European Union (Regulation No 396/2005 and its amendments [2]).

Acknowledgments: We'd like to thank to our beloved colleague Maria Graça Melo (retired) for the extraordinary work in the development of analytical methods for pesticides residues in fruits and vegetables.

References:

[1] SANTE (2019). Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANTE/12682/2019.

[2] EU (2005). Regulation (EC) No 396/2005 of the European Parliament and the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Official Journal of the European Union L70/1-16.

[SBE 15]

Multiresidue Analysis of Pesticides Residues in Potatoes by QuEChERS Sample Preparation and HPLC-MS/MS Analysis

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Solanum tuberosum L., commonly known as potato, is a root vegetable that is widely used for culinary purposes. Potatoes are a good source of different vitamins and minerals, particularly potassium and vitamin C.

Pesticides are used to destroy or repel pests, fungi, insects, and crops' diseases during their growth. The global market for these chemical compounds is expanding firmly. In this line, the European Union (EU) decided to set maximum residue levels of pesticides in or on food and feed of plant and animal origin (Regulation (EC) No 396/2005 [1] and its amendments) to control their use and minimize their potential adverse side effects.

This study aims to validate a simple, economic, and reliable method to determine pesticides residues in potatoes, as a representative matrix for the root and tuber vegetables group.

For the extraction of the pesticides' residues, QuEChERS method was used due to be Quick, Easy, Cheap, Effective, Rugged, and Safe, as the name indicates. The extract was analyzed by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). A total of 145 pesticides were validated, according to the guidance document SANTE/12682/2019 [2]. The limit of quantification (LOQ) was 5 mg/kg for all the pesticides, except for pirimiphos-ethyl, imazalil, and buprofezin (LOQ 10 mg/kg). The method was linear in the range 5-50, 5-60, or 5-70 mg/L, depending on the pesticide, except for buprofezin and pirimiphos-ethyl. The method was proven to be accurate and precise. Accuracy was evaluated through recovery assays and it was between 78-107% and the RSD between 3.7-13.6% for the spiking levels tested (5, 10, and 50 mg/kg). The method showed efficacy and demonstrated to be adequate for monitoring a vast range of pesticides in root and tuber vegetables (including sugar beet, carrots, sweet potatoes, besides potatoes), according to the Regulation (EC) No 396/2005 and its amendments.

Acknowledgments: We'd like to thank to our beloved colleague Maria Graça Melo (retired) for the extraordinary work in the development of analytical methods for pesticides residues in fruits and vegetables.

References:

[1] EU (2005). Regulation (EC) No 396/2005 of the European Parliament and the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Official Journal of the European Union L70/1-16.

[2] SANTE (2019). Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. SANTE/12682/2019.

[SBE 16]

Multiresidue Multiclass Method for the Extraction and Detection of Pesticides and PCB's in muscle

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Pesticides have been extensively used around the world to help the development of agricultural productivity through the management of a wide range of pests and diseases. However, it is well known that the use of these drugs can have severe adverse effects in both human health and the environment [1].
The development of fast multi-residue multiclass methods, allowing the determination of a variety of active substances in a single injection, is a preference, but, at the same time, it is still a challenge for researchers. The great diversity of physicochemical properties of these drugs is an obstacle to achieving an excellent analytical performance for a large number of molecules analyzed simultaneously. New sample preparation techniques continue to be developed to achieve a compromise between good recoveries and interference-free extracts [2].
In accordance with Regulation (EU) No. 2017/625 [3], Member States of the European Union must establish an annual monitoring plan for various pesticide residues in animal matrices, including organochlorines, organophosphates, pyrethroids, carbamates and polychlorinated biphenyls (PCBs) in the muscle matrix. Therefore, a methodology for the analysis of these compounds in the muscle matrix was optimized and validated.
Total fat (2-7%) was obtained by adding anhydrous sodium sulphate to 10g of muscle and left at 50 ºC.The step of extraction was performed using n-hexane saturated acetonitrile solution with lipid freeze-out and a clean-up using SPE Alumina columns (6cc, 1g). In the end, the final extract was split into two, one to be performed in a gas chromatography system coupled to a mass detector and the other in a high-performance liquid chromatography system coupled to tandem mass spectrometry.
The validation procedure for both screening and confirmation was based on document SANTE/12682/2019 [4]. Good results were obtained for 83 pesticides and 7 PCB's with coefficients of variation lower than 20% and average recovery ranged between 70-120%.

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[SBE 17]

In vitro Bioaccessibility of Food Packaging Contaminants of Emerging Concern in Foods: a Tool for Human Dietary-Exposome Assessment – BACFood4Expo

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Human dietary exposure to chemicals is a priority issue for public health authorities and constitutes a key step in risk evaluations.
The safety of food contact materials, such as food packaging, is a main topic in the field of food safety in the EU and requires the evaluation of the chemical substances that can migrate from the material into the food. Among the potential migrants that can be found in packaging materials, the Endocrine disrupting compounds (EDCs) remain a group of chemicals with implications in the occurrence of metabolic diseases with a high prevalence such as Diabetes and Obesity [1, 2]. The safety evaluation of these chemical substances that could migrate into food and be absorbed in the gut is very important from a toxicological point of view since foodstuffs undergo a series of processes before being absorbed into the body, such as the gastrointestinal digestion.
BACFood4Expo project intend to address the problem of chemical contamination of food from packaging materials and contribute with relevant information related with the bioaccessibility of chemicals substances and their potential degradation products during the gastrointestinal digestion after the oral intake to assess the impact of human dietary exposure and contribute to their risk assessment.
The in vitro bioaccessibility aproach proposed, will provide new insights on dietary exposure through the consumption of foods in the Spanish population and may be valuable to help consumers to make wiser food choices, as well as it will enable food safety and health authorities to integrate this information in risk assessment and communication activities.
The BACFood4Expo project findings will also contribute to ensuring the distribution of healthy and safe foods along the food supply chain with economic and social implications since the consumption of healthy and safer foods reduce risk of diseases and therefore reduce the economic costs of national healthcare institutions.

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[SBE 18]

Herbs with adaptogenic properties: a natural solution to stress and anxiety

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Portugal is one of the European countries with the highest prevalence of psychiatric disorders, including anxiety, impulse control and substance use (22.9%), these being more frequent in the group between 18 and 34 years old [1]. Adaptogenic herbs have long been used in traditional Chinese medicine and in the Indian healing practice of Ayurveda. The aim of this work is to assess the novel food status, scientific and regulatory issues for health claims of adaptogens from natural origins, and in parallel conduct a market study of existing products.
Adaptogens are phytochemical substances found in certain herbs and plants that can help improve our ability to cope with stress. The activity of adaptogens is associated with the regulation of homeostasis at system level via several mechanisms of action that are linked to 1) the hypothalamic-pituitary-adrenal axis and at cellular level via activation of molecular chaperones, mainly hsp70 proteins; 2) regulation of key mediators of the stress response, including neuropeptide Y (NPY), cortisol, nitric oxide, stress-activated protein kinase JNK, and forkhead-box transcription factor DAF-16; and 3) the biosynthesis of ATP, which changes the energy source [2-3]. Adaptogenic plants most commonly used in new launches, in the food and drink categories of European market (Mintel database), since 2016, include turmeric and curcumin (91%), goji berry (5%), ginseng (2%) and maca (1%), among several others such as holy basil, and extracts of rhodiola, schizandra and ashwagandha root. Prepared meals, sauces and seasonings, sugar and gum confectionery, and snacks are the main food categories for new products launches, although supplement capsules may be one of the easiest ways to integrate adaptogens into the diet. They can also be combined with cacao and other ingredients in a powder for a soothing drink, or taken as an herbal tea, potentially enhancing the stress-reducing properties of the beverage.

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[SBE 19]

Assessment of regulated mycotoxins in maize harvested in Portugal

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Mycotoxins are secondary metabolites produced by the genera *Aspergillus*, *Penicillium*, *Claviceps*, *Alternaria,* and *Fusarium*, which belong to the most relevant contaminants of food and feed [1]. The incidence of mycotoxins in maize grains is a main concern for human and animal health, resulting in acute or chronic consequences such as carcinogenic, teratogenic, immunosuppressive, or estrogenic effects [2]. The information of mycotoxin occurrence in maize produced and commercialized in Portugal is still limited.

The aim of the present study was to investigate the occurrence of mycotoxins in maize grains harvest in Portugal during 2018 – 2020 period. A hundred and eight maize samples collected in Tagus Valley region of Portugal were analysed by Chemiluminescent immunoassay [3] and UHPLC-ToF-MS [4] for quantification of mycotoxins regulated by Reg. EC No. 1881/2006 [5]. Despite the screening of mycotoxins produced by other genus of fungi (e.g *Aspergillus*) were only found metabolites produced by *Fusarium*, the fumonisins (Fum B1 and Fum B2) and deoxynivalenol (DON). Fum B1, Fum B2 and DON were quantified in 53.7%, 41.7% and 52.7% of samples, respectively. The maximum levels of Fum B1, Fum B2 and DON found in the analysed maize samples, were 2743.2 µg/kg, 1009.5 µg/kg and 484.1 µg/kg, respectively. In total of samples analysed only 3.7% of the samples registered levels of Fumonisins (B1+B2) higher than 2000 µg/kg, and 34.5% of samples showed DON values less than 300 µg/kg. The sum of Fum B1 + Fum B2 and DON levels were below the European Union permitted maximum levels in all maize samples analysed [5].

In conclusion, our results contributed to increase the knowledge on mycotoxin contamination of maize grain produced in Portugal. Additionally, for controlling mycotoxin contamination of maize grains is crucial to adopt good agricultural practices during growth crop period, transport, and storage.

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[SBE 20]

Characterization of the antioxidant properties of green tea (*Camellia sinensis* L.) produced in Mainland Portugal (Oporto Region)

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Tea is a popular beverage obtained by immersing the leaves, buds, or stems of the *C. sinensis* L. plant in hot water [1]. Compared to other teas, green tea is particularly rich in antioxidants due to a manufacturing process that involves drying techniques, which will lead to the inactivation of polyphenol oxidase activity, preserving the active substances by preventing any oxidation reaction from occurring [2].
Portugal is one of the few producers of tea (*C. sinensis* L.) in Europe. Until recently, Portugal only had green tea plantations in its islands; however, aiming to produce high-quality organic green tea, the "Chá Camélia" company started to produce it, in mainland Portugal, on a farm near Vila do Conde. This new green tea has never been characterized before.
Thus, the main objective of this study was to characterize the antioxidant properties of "Chá Camélia" green tea from 2020 production while comparing them to other green tea samples ("Gorreana" and "Happy flora"). The antioxidant capacity of green teas was assessed through DPPH free radical scavenging activity and *β*-carotene bleaching assay. Moreover, total phenolics and total flavonoids content were also determined.
Our results demonstrated that the "Chá Camélia" green tea, produced in mainland Portugal, had excellent antioxidant properties (AAC=600; 39.8 μg T.E./ml; 385.5 mg GAE/ml tea; 113.3 mg ECE/ml tea) compared to the other evaluated samples. Furthermore, it has been shown to have the highest potential to retain the antioxidant capacity in consecutive infusions prepared from the same leaves. In this regard, the retention capacity (calculated between the fourth and the first infusion prepared from the same leaves) was 85% according to the *β* -carotene bleaching assay and 97% by DPPH radical assay.
This study reports the outstanding antioxidant properties of the green tea from the leaves of the Portuguese "Chá Camélia" tea plantation.

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[SBE 21]

Mycotoxins contamination in rice: analytical methods, occurrence and detoxification strategies

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The prevalence of mycotoxins in the environment is associated with potential crops contamination, mostly cereals, which results in an unavoidable increase of human exposure. Rice, being the second most consumed cereal worldwide, constitutes an important source of potential contamination by mycotoxins. Climatic changes are proven to be associated with fungal growth in foodstuffs, leading to increased mycotoxins' contamination.
Due to the increasing number of notifications reported by the Rapid Alert System for Food and Feed (RASFF) portal of the European Commission [1], and the publication of studies that reported the occurrence of mycotoxins in levels above the legislated limits, this work intends to compile some of those studies and review the main methods used on the detection and quantification of these compounds in rice. Moreover, the toxic effects associated with mycotoxins contamination were also reviewed, along with the techniques used in order to attempt to minimize that contamination.
The aflatoxins and ochratoxin A are the predominant mycotoxins detected in rice grain (brown, white) and also in rice flours and these data reveals the importance of adopting safety storage practices that prevent the growth of producing fungi from Aspergilus genus along all the rice chain [2-4].
Further investigation is still required to establish the real exposition to these contaminants, as well as the consequences and possible synergistic effects due to the co-occurrence of mycotoxins and also for emergent and masked mycotoxins.

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[SBE 22]

Use of moderate electric fields for the extraction of bioactive and texturizing food ingredients from macroalgae: a biorefinery approach to clean label foods

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With the increase in population and, subsequently, in food demand, the use of novel foods and ingredients is fundamental. Seaweeds are promising alternatives for the food industry due to their availability, nutritional value, wide range of macro and micronutrients and high added-value bioactive compounds [1,2]. Of equal importance is the use of more environmentally friendly processing strategies. The use of moderate electric fields is a sustainable alternative, recognized for its energy efficiency and reduced emissions, with given proof in the extraction and functionalization of algae fractions [3,4,5].
In order to fully valorise the red seaweeds Gracilaria vermiculophylla and Porphyra dioica into functional food ingredients, a series of sequential extraction procedures was applied: i) aqueous extraction (aimed at phycobiliprotein recovery); ii) ethanolic extraction (targeting chlorophyll and carotenoid recovery) and
iii) hot-water extraction (for gelling polysaccharide recovery). Moreover, the effect of green alternatives (moderate electric fields) on the recovery yields and behaviour of the extracted fractions was accessed.
The use of moderate electric fields allowed for an increase in the extraction yield of phycoerythrin (1.6 times), without compromising its thermal stability, alongside an increase in chlorophyll a (1.3 times) and chlorophyll b (1.8 times). Furthermore, the extracts obtained using electric fields presented a higher antioxidant activity than those obtained by conventional heating. Regarding the texturizing fractions, the use of moderate electric fields increased the gelling strength 1.3 times, without compromising the extraction yields. Lastly, the remaining solid fraction was further valorised using enzyme-assisted extractions aimed at aimed at bioactive peptide and fermentable monosaccharide recovery.
Overall, the used strategy proved that it is possible to recovery functional and bioactive ingredients from red seaweeds, using more energetically sustainable processes, that can be applied as clean-label colouring and texturizing agents, alongside nutraceutical and bioactive ingredients, towards more nutritious and natural food alternatives.

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[SBE 23]

Spent coffee grounds as a food byproducts source of polysaccharide with application as carriers for pulmonary insulin delivery

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Polysaccharides are often found in considerable amounts in byproducts from food industry, where they lose their food properties, but become innovative components for other applications. Spent coffee grounds (SCG), resultant after espresso coffee preparation, are a polysaccharide-rich source, namely for galactomannans (GM) and arabinogalactans (AG). Due to the biocompatibility and biodegradability of GM and AG, these polysaccharides are promising vehicles for insulin delivery. Being a protein, insulin is highly susceptible to degradation during powder preparation, thus requiring carriers. Insulin as powder may be administered to diabetics via the pulmonary route, an alternative to subcutaneous injections [1]. In this work, microparticles based on GM and AG to carry, protect, and deliver insulin were developed. GM and AG were extracted from the SCG by Microwave Assisted Extraction (MAE) with temperatures of 150 ºC and 180 ºC, respectively [2]. For comparison purposes, GM were also obtained from locust bean gum (LBG). Due to the high viscosity of LBG, this sample was partially hydrolyzed in subcritical conditions also using microwaves. The high molecular weight fractions from the SCG extractions were separated by ultrafiltration (5 kDa). All samples were assessed for their sugar composition and spray dried with insulin (10% w/w). The resultant microparticles were assessed for their size and morphology using scanning electron microscopy. Insulin release kinetics were evaluated by HPLC. Sugar analysis confirmed GM- and AG-rich fractions. All microparticles presented raisin-like morphologies with sizes between 1-5 µm, a prerequisite to achieve alveolar deposition. Insulin was release from GM-based microparticles in a gradual and continuous manner independently of source (SCG or LBG), while AG- based microparticles released insulin in a sigmoid manner. These results suggest that insulin release profile is dependent on polysaccharide-type rather than source. These results show that polysaccharides extracted from byproducts to avoid food competition, can be used in the development of pharmaceutical formulations.

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[SBE 24]

Antibiotic Resistance Profile among Shiga Toxin-Producing *Escherichia coli* Isolated from dairy cattle

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Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that can cause serious diseases in humans, including bloody diarrhoea and kidney failure. Ruminants, such as cattle, are considered the main reservoirs and source of STEC. Human infection can occur through contaminated food and water, or direct contact with infected animals. STEC antimicrobial-resistance (AMR) is increasingly frequent in patients with serious disease. It is necessary to understand the epidemiology, the emergence, and the prevalence of AMR in STEC isolated from cattle to investigate how resistance spreads from ruminants to humans. Thus, susceptibility tests were performed on 55 STEC strains belonging to 29 serogroups. The strains were isolated from healthy dairy cattle faeces (cows and heifers) in the North of Portugal. Antibiotic susceptibility testing (AST) was performed by disc diffusion method following European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020) and Clinical and Laboratory Standards Institute (CLSI, 2020). The antibiotics used included penicillins (ampicillin; amoxicillin-clavulanic acid), cephalosporins (ceftazidime; cefotaxime; cefoxitin; cephalothin), carbapenems (imipenem; meropenem), aminoglycosides (kanamycin), phenicol (chloramphenicol), sulphonamides/ trimethoprim (trimethoprim-sulfamethoxazole), fluoroquinolones (moxifloxacin; levofloxacin) and tetracyclines (tetracycline; tigecycline). Results reveal low level of resistance among the isolates tested. However, five (9%) STEC isolates were resistant to one antibiotic, and three (5,5%) to three or more antibiotic classes (multidrug resistance-MDR). The MDR strains were resistant to trimethoprim-sulfamethoxazole, tetracycline and other antibiotics commonly used to treat gastroenteritis. Two strains MDR belonged to O91 serogroup and were founded in heifers in the same farm. O91 is an important serogroup to public health surveillance, as it is commonly associated with contamination of products from animal origin, and it has been isolated from patients with severe gastrointestinal disease. Overall, the AMR did not seem to be widely spread in STEC isolates from cattle; but serotype O91 might be of special concern as two O91-multidrug resistance profiles have been identified.

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[SBE 25]

Optimization and validation of an analytical methodology for mycotoxin determination in maize grain by a QuEChERS-based UHPLC-MS/MS multi-method

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Mycotoxins are toxic compounds of low-molecular weight that occur in every step of the agri-food chain due to its ability to bioaccumulate through different levels of the food pyramid [1]. Its negative effects have impact in harvest, production profitability, animal health and safety of end-consumer products [1,2]. Since maize is one of most prominent cereal crops in the world, presenting a key role in food security and sovereignty, its quality and safety as an animal and human food product must be ensured [3]. Mycotoxin monitoring is the first step of an accurate risk assessment and, consequently, analytical procedures must be well established to guarantee reliable results.

This study was focused on the optimization and validation of an analytical methodology by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for the determination of mycotoxins in maize grains. Determination of fusariotoxins, Penicillium, Aspergillus and Alternaria toxins, as well as emerging toxins was firstly optimized by testing several analytical extraction approaches, including Solid-Liquid Extraction, Solid Phase Extraction and QuEChERS. Development and validation of the analytical method using the best extraction procedure was latter performed. In this matter, blank samples fortified with mycotoxin standards were subjected to a modified QuEChERS protocol, using a combination of C18 and PSA sorbents as purifying agents for dispersive SPE. Validation parameters, including linearity, limit of detection (LoD), limit of quantification (LoQ), precision and recovery were evaluated. Performance criteria was performed according to specific requirements for confirmatory methods stated in Commission Regulation nº 401/2006 [4]; and non-regulated mycotoxins followed the guidelines established by FDA [5], ICH Q2 (RI) [6] and Relacre [7].

The present methodology successfully allowed the extraction and determination of 23 mycotoxins in maize grains, by providing great specificity and linearity for all the compounds, and an unequivocal detection and confirmation of all mycotoxins analysed at low levels in this matrix.

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[SBE 26]

QuEChERS-based UHPLC-MS/MS multi-method for the determination of emerging and regulated mycotoxins in samples from maize agricultural crops and dairy farms

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Maize (Zea mays L.) is a highly valuable agricultural and industrial product in worldwide food supplies, especially in the current challenging global scenario of providing a stable and safe food supply compliant with an increasing population rate[1]. This agricultural cultivar is used in human diet in various parts of the world, mainly in underdeveloped countries, and as a major feed component for livestock, with a high nutritional and economic value for all age groups[2]. Therefore, mycotoxin contamination of this cultivar and, subsequently, of its end-products, negatively impacts its quality and safety as an animal and human food product[3]. The presence of mycotoxins in these food chain needs constant monitoring to evaluate the risk to which each population group is exposed to.
A new analytical methodology based on QuEChERS extraction with a combination of C18 and PSA sorbents, followed by UHPLC-MS/MS analysis for the determination of mycotoxins in maize grains was extended to standardize a unique methodology encompassing several matrices from the maize value chain, including seeds, flowering plants, silage, and animal feed. Matrix effects were monitored for these matrices regarding the maize value chain, for emerging (enniatins, beauvericin, and moniliformin) and regulated (aflatoxins, ochratoxin A, zearalenone, fumonisins, T-2 and HT-2 toxins) mycotoxins.
The method was validated for the referred matrices proving to be specific and selective, with low matrix interferents or peaks that could co-elute with the target compounds, and, therefore, low ionic suppression effects. Good performance criteria were also obtained regarding linearity, repeatability, reproducibility, and recovery, in compliance with Commission Regulation (EC) Nº 401/2006[4] and Commission Regulation (EU) Nº 519/2014[5] for regulated mycotoxins. LODs and LOQs demonstrated the method's capacity to determine concentrations below the maximum residue levels for such samples, as established in Commission Regulation (EC) Nº 1881/2006[6].

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[SBE 27]

Coffee: how to tailor its cholesterol-lowering activity?

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Coffee brew is composed by several bioactive compounds such as polysaccharides (galactomannans and arabinogalactans), which have been shown to have hypocholesterolemic potential[1]. One of the main mechanisms by which polysaccharides are known to affect cholesterol absorption at the intestine is their ability to sequester bile salts, lowering cholesterol bioaccessibility[2]. Coffee processing parameters such as degree of roasting (DR) and grinding level (GL) have been shown to affect these polysaccharides' structure and content[3,4]. However, it is not yet described how these changes can influence coffee hypocholesterolemic activity. In this work, coffee was subjected to three different DR (light, medium and dark) and two GL (fine and coarse) and characterized regarding its sugar composition. The brewed coffees alone and supplemented with galactomannans and arabinogalactans rich fractions were then evaluated towards their effect on cholesterol solubility in an *in vitro* intestinal model composed by cholesterol and bile salt[1].
The different DR and GL highly affected the hypocholesterolemic properties of the brews. Overall, cholesterol bioaccessibility decreased with the increase of the degree of roasting and for coarser grinding, being the ranges obtained of 38-48%, 46-49% and 31-54% for light, medium and dark coffees, respectively. These values were compared with a cationic resin (pharmaceutical control) which reduces 81 % of cholesterol bioaccessibility. As for polysaccharides rich fractions obtained from light roasted coffee, the cholesterol-lowering activity of arabinogalactans (26%) was greater than that from galactomannans (10%). Light roasted coffee enriched with the polysaccharide fractions was also evaluated, being the one enriched with arabinogalactans more effective towards cholesterol reduction (49%) than the one enriched with galactomannans (44%).
The results of this work showed that by modulating coffee processing parameters it is possible to improve its hypocholesterolemic properties, in order to develop tailor-made preparations with cholesterol-lowering potential.

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[SBE 28]

Differences in antimicrobial activity of natural compounds by drop diffusion or dilution methods on agar

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The interest for natural antimicrobials as an application in food preservation has been increasing due to the growing interest of the population for a healthy lifestyle. Natural compounds have interesting characteristics, such as biodegradability and biocompatibility [1], making them an alternative to chemical compounds in conservation. Thus, it is important to carry out screening methods to identify the antimicrobial activity of these compounds. The *in vitro* determination of the antimicrobial activity of natural compounds requires determining their minimum inhibitory concentrations to assess microbial susceptibility.
This study aimed to evaluate the minimum inhibitory concentrations of three antimicrobial potential natural compounds – chitosan, ethanolic propolis extract, and nisin – against 37 microorganisms by agar dilution and drop diffusion on agar methods. Antimicrobial activity was tested in culture media at different pH values to simulate different food products. Most Gram-positive bacteria were inhibited at 25 µg/mL of nisin, and most of the microorganisms were inhibited by chitosan at 0.5% (w/v) and propolis at 10 mg/mL. The inhibitory action of the compounds was influenced by the evaluation methods and pH values. In this study, it was concluded that, in general, lower minimum inhibitory concentrations were observed at lower pH values and for the agar dilution method. Some microorganisms inhibited by the compounds on the agar dilution method were not inhibited by the same compounds and at the same concentrations on the drop diffusion technique. Therefore, this study reinforces the need for using a defined standard method for the *in vitro* determination of minimum inhibitory concentrations of natural compounds because it is crucial to compare results obtained in different studies and matrices.

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[SBE 29]

Clean label meat products: Natural nitrate sources & nitrate-reducing starter cultures

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"Clean labelling is no longer a trend but rather a consumer demand" [1]. Being an important curing agent in meat products, with roles in antioxidant activity, antimicrobial effects and sensorial characteristics, replacement of nitrite by a single alternative ingredient is challenging [2]. This study aimed to evaluate a nitrate reductase-producing starter culture and natural sources of nitrate as a potential clean label solution to nitrite addition in cooked ham manufacturing.
Cooked hams were prepared at a laboratory scale using minced pork leg, water, salt, sodium tripolyphosphate, and carrageenan. A preliminary test was conducted to evaluate the performance of the culture to reduce added nitrate into nitrite. Then, the possibility of using this starter and vegetable sources of nitrate was investigated. To the base formulation was added: a) starter culture, b) sodium nitrate ($NaNO_3$: 30 to 300 mg/kg) in combination with the starter culture, c) dried beetroot and paprika with the starter culture and d) nitrite (1 g/kg). After processing, the cooked hams were submitted to several microbiological and physico-chemical analysis.
A typical ham color was observed with the starter culture and 150 mg/kg of $NaNO_3$. Colorations not characteristic of a ham were observed when beetroot and paprika were used to replace added $NaNO_3$. Different texture parameters were determined for hams prepared with nitrite or beetroot and paprika in combination with the starter culture, being both products microbiological stable during at least 5 days at 4 ºC.
Although preliminary, these results demonstrated that 150 mg/kg of $NaNO_3$ was the minimal concentration to develop an acceptable color in hams in combination with the starter culture. Beetroot and radish, at the concentrations used, resulted in non-characteristic color of the matrix under study. More tests should be performed to optimize the use of these vegetables as an alternative to the addition of nitrite in ham production.

Acknowledgments: This work was developed in the scope of the project "cLabel+: Innovative "clean label" natural, nutritious and consumer-oriented foods" (POCI-01-0247-FEDER-046080), co-financed by the Fund European Regional Development (ERDF) through the Competitiveness and Internationalization Operational Program (POCI).

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[SBE 30]

Potential antineurodegenerative properties of bioactive compounds from olive seeds using Ultrasound-Assisted Extraction

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This work aimed to evaluate the phytochemical composition of olive seeds extracts from different cultivars (Cobrançosa, Galega and Picual), as well as their antioxidant capacity. In addition, it also intended to appraise the potential antineurodegenerative properties through the ability to inhibit enzymes associated with neurodegenerative diseases: acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase (TYR).
To achieve this goal, the phytochemical composition of these samples was performed through spectrophotometric (total phenols, flavonoids, and *ortho*-diphenols) and chromatographic methodologies (HPLC–DAD–ESI/MS^n). The antioxidant capacity was performed by two different methods (ABTS and DPPH) and the antineurodegenerative potential by the capacity of these extracts to inhibit related enzymes: acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase (TYR).
The results showed that seed extracts present a high content of phenolic compounds, and a great ability of scavenging ABTS•+ and DPPH•. The HPLC-DAD with Mass Spectrometry indicated the presence of one phenyl alcohol (tyrosol), two flavonoids (rutin and luteolin-7-glucoside), and three secoiridoids (nüzhenide, oleuropein, and ligstroside). Galega was the most promising cultivar, not only due to its high concentration in phenolic compounds, but also because of its high antioxidant capacity and strong inhibition of AChE, BChE, and TYR.
It can be concluded that olive seeds extracts may provide a new and alternative source of agents for medical and industrial applications.

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[SBE 31]

NOBEL Project: seNiOr meals towards high nutrient BioaccEssibiLity

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The world population is increasingly aging together with an increase in the incidence/prevalence of various comorbidities, largely due to phenomena of malnutrition. In addition, the current SARS-CoV-2 pandemic crisis had a considerable negative effect on the health of the elderly. Therefore, it is essential to ensure the adequacy of primary health care, namely regarding their nutrition.
To ensure an adequate nutritional status of this population, the supply of nutrients must target all the morphological and physiological changes that characterize them, such as the depletion of muscle/bone mass, reduction of mastication/swallowing mechanisms, the digestive efficiency from an enzymatic point of view and pH and even the modification of nutritional requirements. Thus, designing an adequate nutritional plan is as essential as ensuring that all the necessary nutrients are available to be transported through a naturally modified gastrointestinal tract.
The project NOBEL aims to nutritionally characterize the macro and micro components of ITAU-Senior meals, demonstrating their adequacy to the needs of the target population. Then, “in vitro” monitoring of selected micronutrients will be carried out through harmonized human digestion protocols adapted to the geriatric population. This innovative approach will allow us to intervene in meeting these nutritional requirements.
The outcomes of this partnership between academia and industry will provide a new vision on effective nutrition in the elderly, being extremely relevant to overcome malnutrition, contribute to reducing the impact of chronic diseases and thus promote active and healthy aging.

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[SBE 32]

Raw milk as a source of food zoonotic pathogens

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The growing idea that natural products are better for consuming are creating behaviours that can lead to food safety problems. One of the trend products is raw milk that in some countries is sold by self-service vending machines outside dairy farms [1].

In the present study we have determined the occurrence of *Campylobacter* spp. and *Listeria monocytogenes* in raw milk. Campylobacteriosis is the most commonly gastrointestinal infection in humans in the EU since 2005 [2], while listeriosis is one of the most serious food-borne diseases under EU surveillance causing high hospitalisation and high mortality, particularly among the elderly [3]. Several outbreaks have been associated with the consumption of raw milk contaminated with either *Campylobacter* spp. or *L. monocytogenes* [4] [5].

The study was conducted over one year, from November 2020 to September 2021, on randomly selected dairy farms located in the north of Portugal. One litre of milk was collected from bulk milk tank transported to the laboratory and were analysed within 24h. Detection of the pathogens was performed using real time PCR methodology and confirmation followed ISO standards. From 100 dairy farms evaluated, the occurrence of *Campylobacter* spp. was estimated in 4% while *L. monocytogenes* was not detected in any sample.

The results of this study indicate the potential risk of campylobacteriosis after the consumption of raw milk. Consumers seeking for raw milk due to health reasons should be aware of the risk, especially if belonging to vulnerable groups.

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[SBE 33]

Lactic Acid Bacteria isolated from "innovative" alheiras as potential biocontrol agents

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The use of no-pathogenic bacteria and/or their metabolites as natural antimicrobials has been a hot topic due to their capacity to enhance food safety and shelf-life extension (1).
In this study, three Lactic Acid Bacteria (LAB) isolated from "innovative" *alheiras* and belonging to the species *Leuconostoc mesenteroides* (4-8 - codfish *alheira*), *Lactobacillus curvatus* (9A3 - vegetables *alheira*), and *Pediococcus acidilactici* (10A2 - mushrooms and shiitake mushrooms *alheira*) were characterized regarding their safety and beneficial features. Virulence factors such as gelatinase, DNAse, haemolytic activity, and biogenic amines production were investigated, as well as antibiotic susceptibility and the presence of genes encoding virulence factors. Additionally, the antimicrobial activity of each strain against several foodborne pathogens was also verified and characterized (2).
All strains lacked the virulence factors investigated but harboured some virulence genes. However, the presence of some genes alone does not necessarily mean that the strain is virulent (3). Related to antibiotic susceptibility, only *Lact. curvatus* 9A3 was sensitive to all antibiotics tested. Furthermore, antimicrobial activity against several foodborne pathogens was observed as well as bacteriocinogenic activity against several strains of *Listeria monocytogenes* was found for all the strains. The higher bacteriocin activity (12800 AU/ml) was observed for *Lact. curvatus* 9A3, while *Leuc.mesenteroides* 4-8 and *P. acidilactici* 10A2 produced bacteriocins with lower activities (100 AU/ml) against the *L. monocytogenes* tested strains.
The studied LAB showed essential attributes, such as the absence of important virulence factors and genes, and the production of proteinaceous compounds with antimicrobial activity against the foodborne pathogen *L. monocytogenes*. This indicates that "innovative" *alheiras* are also a source of LAB with appealing characteristics for the food industry. Further tests are required, but the LAB studied, and the strain *Lact. curvatus* 9A3 in particular, seem to be promising candidates to be used in future biocontrol approaches.

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Dare 2 Change

Sustainability

[SUS 1]

The seaweed as resources to produce active edible films

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Seaweeds (also called marine algae, macroalgae or "sea vegetables") are aquatic photosynthetic organisms that have been worldwide explored mainly for food purposes due to their great nutritional profile.[1] Within the algae compounds, the polysaccharides, namely agar, carrageenan, and alginate have been also used commercially as thickeners, gelling agents, and stabilizers.[2] More recently, these seaweeds' polysaccharides have been proposed for food packaging and coating materials application.[3] Thus, the aim of this study was to develop edible films to be applied in food products using the carbohydrates extracted with water from the red seaweeds *Gracilaria gracilis* (Figure 1a) and *Chondrus crispus* (Figure 1b), and the brown seaweed *Bifurcaria bifurcata* (Figure 1c).

The three algae were washed, dried at 50 °C until constant weight, and milled. An aqueous extraction of the powders was performed to obtain carbohydrate-rich extracts. *G. gracilis* extract comprised 64% of carbohydrates, mainly constituted by galactose (45% molar) and 3,6-anidrogalactose (31%), inferring the presence of agar. The extract of *C. crispus* contained 44% of carbohydrates, mainly galactose (75 % molar) and 3,6-anidrogalactose (16%), confirming the presence of carrageenan. On other hand the extract of the brown algae *B. bifurcata* was composed by 62% of carbohydrates, mainly composed by uronic acids, in accordance with the presence of alginate.

To obtain the edible films, the extracts were dissolved in distilled water (1% w/v) and glycerol (0.3% w/v) was added into the solutions as plasticizer. The solutions were filtered under vacuum, degassed, and transferred into a plexiglass plate. The plates were placed in an oven for 15 h at 35 °C for film formation by solvent casting. The films produced from *B. bifurcata* extracts were immersed in a calcium alginate solution (2% w/v) for 3 min to increase their water resistance. The extracts obtained from the different algae were able to form films with antioxidant properties (Figure 1d, e, and f). The *B. bifurcata* and *C. crispus* based films revealed an inhibition of 93% and 81%, respectively, after 8h of reaction (measured by ABTS method), while *G. gracilis* based films presented a lower inhibition of 42%. Nevertheless, all extracts obtained from the different algae revealed a great potential to develop edible films with antioxidant capacity to be applied in food industry.

Figure 1: Seaweeds: a) Gracilaria gracilis, b) Chondrus crispus, and c) Bifurcaria bifurcata. Seaweeds based films of: d) Gracilaria gracilis, e) Chondrus crispus, and f) Bifurcaria bifurcata.

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[SUS 2]

HS-SPME/GC×GC-ToFMS as tool to identify volatile compounds markers of PDO "Pera Rocha do Oeste"

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The "Pera Rocha do Oeste" pear, due to its sui generis characteristics, namely its aroma, is an important Portuguese Protected Designation of Origin (PDO) [1] product, and its authenticity has an added economic value. To identify authenticity markers based on volatile compounds, it is important to select only the contribution of compounds that do not vary according to the storage conditions of the fruits. Aromatic compounds with description of pear and fruity odor, such as esters, alcohols, aldehydes and terpenes, are influenced by the environmental conditions of the orchards and fruit storage [2,3].
In this study, a method based on headspace solid-phase microextraction (HS-SPME) coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-ToFMS) [4] was used to analyze volatile compounds of PDO "Pera Rocha do Oeste" from different orchards stored in different atmosphere and of pears from Alentejo.
From a total of 130 volatile compounds, 42 were identified with pear or fruity odour descriptor, mainly esters (28), followed by alcohols (9), aldehydes (3), and terpenes (2). From these 42 compounds were 14 selected compounds (1-butanol, 1-hexanol, ethyl acetate, butyl acetate, pentyl acetate, butyl butanoate, ethyl hexanoate, hexyl acetate, heptyl acetate, ethyl octanoate, hexyl hexanoate, ethyl 2,4-decadienoate and α-farnesene). On the one hand, the profile and the relative abundance of the selected compounds showed little variation between pears harvested in different orchards in the PDO region (9) stored in different atmosphere conditions (normal and controlled). On the other hand, these 14 compounds make it possible to separate PDO pears and pears from Alentejo. The 14 selected compounds can be proposed as markers to track the authenticity of the PDO "Pera Rocha do Oeste". The HS-SPME / GC × GC-ToFMS methodology is an excellent tool to identify the characteristic compounds of the fruits and to authenticate the origin of Rocha pears.

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[SUS 3]

Authenticity of PDO "Pera Rocha do Oeste" and PGI "Maçã de Alcobaça" cake fillings by Multielement analysis

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The designation of a food product as Protected Designation of Origin (PDO) [1] or Protected Geographical Indication (PGI) [2] is a guaranty of authenticity and traceability. PDO "Pera Rocha do Oeste" pear and PGI "Maçã de Alcobaça" are important Portuguese products due to its economic value to Portugal.

Apple and pear fruits have their consumption in fresh, as well as industrially processed, as constituents of cake fillings. Although fresh fruits are easy to identify, their processed products require the identification of unique characteristics of the target products, which may serve as markers of authenticity.

It is possible that the fruits of a region possess characteristics associated to the environment [3], conferring them these required unique characteristics for their identification, even when processed.

In this study, the mineral profiles of PDO "Pera Rocha do Oeste" pear and PGI "Maçã de Alcobaça" apple var. Golden Delicious (fresh fruit and fruit fillings) were identified to evaluate the applicability of multielement data on the determination of geographical origin and authenticity. The elements of fresh fruits and fruit fillings were analysed by ICP-MS and ICP-OES after microwave digestion. The results show that PDO pears and pears from the other areas are discriminate by 13 elements. These selected elements, 4 elements (Mn, Ce, B and Rb) are significatively different between PDO pear fillings and pears fillings from Alentejo. In case of apple, PGI fresh apples and fruit fillings have lower caesium (Cs) and rubidium (Rb) concentrations than apples and fruit fillings from other areas. These differences can be explained by the soil characteristic.

The present study shows that multielement analysis combined with the appropriate statistical tools can be a valuable contribution for the identification and authenticity of the geographical origin of pear and apple fillings, even if industrially processed.

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[SUS 4]

Solvent and temperature influence on the microwave-assisted extraction yield of rice bran oil from three varieties of Carolino Rice

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Rice bran is a good source of nutrients such as phytochemicals and antioxidants. Microwave-assisted extraction (MAE) is as a green and effective method for rice bran oil extraction [1]. This work aimed to study the effect of solvents and temperature on the yield of crude rice bran oil microwave-assisted extraction (MAE) from three varieties of Portuguese Carolino rice. The variables tested were extraction temperatures (80, 100 and 120 ºC), solvents (ethanol and propanol), and Carolino rice varieties (Luna, Teti and Ariete). Stabilisation of the rice bran was executed as described by Dauda et al. (2017) [2] and Malekian et al. (2020) [3]. A sample-solvent ratio of 1:9 was used. Results indicate that the main factor for yield differences was the temperature, followed by the solvent. Both solvents perform better at higher temperatures, ethanol leading always to higher yields. The rice variety did not influence the extraction yield ($p>0.05$).

Table 1 – Extraction yields of rice bran oil from three Carolino rice varieties

Solvent	Extraction temperature (ºC)	Rice varieties		
		Ariete	Teti	Luna
Ethanol	80	7.49 ± 1.21^{d}	8.36 ± 0.91^{e}	7.54 ± 0.94^{c}
	100	11.72 ± 0.96^{c}	12.91 ± 0.81^{c}	12.03 ± 1.75^{b}
	120	17.90 ± 1.19^{a}	18.20 ± 1.50^{a}	17.88 ± 1.78^{a}
Propanol	80	6.94 ± 0.24^{d}	7.54 ± 0.38^{e}	6.98 ± 0.71^{c}
	100	9.99 ± 0.95^{c}	10.95 ± 0.90^{d}	10.29 ± 0.92^{b}
	120	16.11 ± 0.88^{b}	16.02 ± 1.10^{b}	17.27 ± 0.23^{a}

Values in the same column with different superscripts are significantly different ($p<0.05$).

It can be concluded that ethanol, an environmental-friendly solvent, is suitable for rice bran oil MAE extraction.

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[SUS 5]

Repurposing potato chips byproducts as additives for developing antioxidant and UV-protective starch-based films

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Food industry byproducts promote environmental and societal concerns. Under a circular economy concept, these byproducts can be valued as a source of biomolecules [1,2], such as polysaccharides, proteins, lipids, phenolics, and high-molecular weight brown colour compounds (melanoidins), with interest to develop active biobased materials. Potato chips industry gives rise to byproducts, such as starch-rich washing slurries and brownish frying residues, that have simply been landfilled, increasing its ecological footprint. Under the field of POTATOPLASTIC copromotion project (POCI-01-0247-FEDER-017938) led by "Isolago", in this work, the brownish-derived extract (BrE) and starch, both recovered from potato chips processing from "A Saloinha, Lda", were *in-situ* gelatinized to form potato starch-based films. The influence of BrE amount (5%, 10%, and 15% w/w of dry starch weight) on optical, mechanical, physicochemical (solubility, wettability), and active (antioxidant and UV-protective) properties of starch-based films was studied.
The incorporation of BrE conferred a yellowish coloration to the starch-based films, while maintaining their transparency, and improved ca. 2x the traction resistance and elasticity of the films. Their wettability increased ca. 15º and 20º the water contact angles at the upper and down films surface, respectively, as well as their antioxidant activity (ca. 94% of $ABTS^{\bullet+}$ inhibition in 4 h). Besides, the films properties were directly related to the BrE dosage: when compared to the pristine films, as high the BrE amount, lower the solubility in water (from 12% in the pristine to almost 0% in films containing 15% of BrE) and higher the UV radiation absorption capacity, mainly at UV-C and UV-A at 340 and 250 nm, respectively, of the BrE/starch-based films.
Therefore, brownish chips-derived extract reveals to contain molecules of interest to tune the performance of potato starch-based films, offering a new *in-situ* strategy to valorise potato chips industry byproducts.

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[SUS 6]

POTATOPLASTIC: Development of biodegradable bioplastics using potato byproducts

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The environmental impact caused by non-biodegradable plastics has led to the search for biodegradable materials, such as bioplastics. Despite of its commercial existence, the industrial production is still reduced due to unsuitable mechanical and physicochemical properties, namely restricted tensile strength and/or flexibility, moisture sensitivity and limited barrier properties, as well as costs that are not yet competitive with those of petroleum-based plastics. As a potential strategy to overcome these constraints and promote bioplastics expansion, POTATOPLASTIC project was designed, supported by Portugal2020 program, aiming the development of biodegradable bioplastics derived from potato byproducts. In this project, starch, oils, and waxes from potato washing waters, frying residues, and potato peels, respectively, were extracted. The combination of starch with oils or waxes allowed to tune the bioplastics performance, yielding transparent, flexible, and hydrophobic materials. POTATOPLASTIC promoted the sustainable development of biodegradable bioplastics, creating the basis for translating the methodology into an industrial scale in the near future.

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[SUS 7]

Valorization of pine nut skin: Microwave-assisted extraction of bioactive compounds

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Pine nut skin (PNS) is a by-product with an annual volume of approximately 550 metric tons worldwide [1]. PNS is easily recovered at the nut processing mill, has low moisture content and low density, reducing the costs associated with drying, transportation, and storage.
To utilize PNS in the industry, low-cost and time-saving extraction methods must be employed. Microwave-assisted extraction (MAE) allows to attain high temperatures and is considered a green extraction methodology, due to the reduction of extraction time, solvent and energy consumption, and the possibility of multiple extractions [2].
In this work, the MAE procedure was optimized to extract phenolic compounds from PNS. A full factorial design was used to estimate optimum extraction conditions, namely temperature (120, 150, 180 °C), time (1, 5.5, 10 min), and the ratio of sample mass to volume (w/v) (1, 2, 3 g to 60 mL of distilled water) on the yield (% w/w), total phenolic content (TPC), and free radical scavenging capacity (ABTS).
The three evaluated responses were significantly affected by the temperature, with a higher yield, TPC, and ABTS being obtained at 180 °C. Besides, the yield was affected negatively by w/v, and by the interaction between time and temperature, with the effect of temperature more noticeable when extraction time was lower. The interaction between time and w/v was significant on ABTS, which increased with time when the w/v was low and decreased with time when the w/v was high. Thus, the condition giving the best results for the three responses, simultaneously, was 180 °C, 1 min, and 1 g, resulting in 18.8 % (yield), 229.1 mg gallic acid equivalent/g skins (TPC), and 310.5 mg ascorbic acid equivalent/g skins (ABTS).
This work demonstrated that PNS could be valorized as a natural source of phenolic compounds, that could be incorporated into food formulations.

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[SUS 8]

TRACE-RICE, Tracing rice and valorizing side streams along Mediterranean blockchain

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The relevance of rice in the European diet has been increasing due to its fundamental role in modern and healthy diets, however rice-based foods are highly prone to adulteration and contaminations (e.g fraudulent variety claims, mycotoxins). Most of the rice consumed in UE is grown in the Mediterranean countries and the TRACE-RICE project address the actual pressing challenges of the rice sector [1].
TRACE-RICE targets the adoption of cost-efficient and environmentally safe tools for traceability, authenticity, contaminant mitigation and conversion of by-products to innovative rice base food produced in the Mediterranean [2]. The main objectives is providing the Mediterranean rice industry with highly efficient and affordable analytical and digital technologies and obtain new nutritional, healthy and high added-value products based on an interdisciplinary integrated chain-wide and circular economy approach. TRACE-RICE pays particular attention on the creation of robust models from unstructured data [3] and implementation of RFID tags for wireless and real time supply chain integration, enabling a more sustainable use of resources and minimizing waste.
The expected environmental impacts of project are the support of contaminant mitigation, reduction of fossil fuels consumption by using blockchain technologies and support the goals of the EU biodiversity strategy by the valorization and genetic characterization of adapted varieties and integration of datasets in a network database. The expected economic impacts are the improvement of the rice quality control, obtention of new high added value products and contribution to the food diversity, promoting Mediterranean cultural heritage and boosting the circular economy. The expected social impacts are foster the Mediterranean rice commercialization in international markets by the mitigation of rice fraud and adoption of measures to reduce the extreme volatility of prices of rice-based foods in the market and facilitate timely access to information thanks to blockchain technologies.

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[SUS 9]

Authenticity markers to distinguish two PDO cheeses: Serra da Estrela and Azeitão

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"Queijo da Serra da Estrela" and "Azeitão" cheeses, due to their sui generis characteristics, are Portuguese Protected Designation of Origin (PDO) products [1,2]. These cheeses are cured cheeses, obtained by slow depletion of the curd after coagulation of raw sheep's milk by the action of the cardoon (*Cynara cardunculus*, L.) enzymes [3,4].
PDO "Queijo Serra da Estrela" cheese is the oldest of all Portuguese cheeses, being internationally recognized for its organoleptic characteristics. It has a smooth, clean and slightly acidic flavor and aroma. The geographical area of production of "Queijo Serra da Estrela" cheese covers several municipalities in the districts of Viseu, Coimbra, Guarda and Castelo Branco [3]. PDO Azeitão cheese has a spicy flavor that is simultaneously acidified and salty. Its organoleptic characteristics are due to the edaphoclimatic conditions in the region, which influence the quality of pastures. The geographical area of production covers the municipalities of Palmela, Sesimbra and Setúbal [4]. As both cheeses are made from sheep's milk, economically it is very important to be able to distinguish the different PDO cheeses.
The present study shows that multielement analysis can be a valuable contribution for the identification and authenticity of the geographical origin of PDO sheep milks from Serra da Estrela and from Azeitão as wells as of PDO cheeses. Also, the methodology based on headspace solid-phase microextraction (HS-SPME) coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-ToFMS) is a tool to identify and quantify the key terpenic compounds, the highland pasture compounds [5], and could be to distinguish "Queijo da Serra da Estrela" cheese from PDO Azeitão cheese.
The present study shows that multielement analysis and HS-SPME / GC × GC-ToFMS methodology are excellent tools to identify the characteristic compounds of the cheeses and to authenticate the origin of different PDO cheeses.

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[SUS 10]

Yeast4FoodMed - Valuation of brewer's spent yeast polysaccharides for food and biomedical applications

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Brewer's spent yeast (BSY) is a major by-product from the brewing industry. Yeast4FoodMed project proposes the valorisation of BSY for novel applications that could emerge from its unique structural features [1]. In this way, valorise BSY as a coproduct should be important to produce yeasts in controlled conditions containing polysaccharides with characteristics suitable to obtain food ingredients, biomaterials, and yeast microcapsules. The BSY microcapsules are obtained after cell wall soluble polysaccharides extraction, where microcapsules preserve their three-dimensional structure being proposed as a delivery system. Through the BSY knowledge will be possible to identify process conditions that modulate yeast, for the expression of molecular structures allowing the proposed applications.

The structural features of BSY cell walls have peculiar glycosidic-linkage compositions, which are induced by the fermentation processes and the adaptation of the yeasts to the alcohol medium [2]. Also, depending on the species used and the number of yeast reutilizations, it is possible to recover different type and structures of polysaccharides for food applications. For example, *Saccharomyces cerevisiae* is a source of (1→3)-glucans or (1→4)-glucans, while *S. pastorianus* is a source of mannoproteins. BSY showed a high variability depending on the yeast strain/species and reutilization.

Yeast4FoodMed project developed food ingredients with emulsifier properties, produced biomaterials with potential application as food packaging, and studied the basis of a universal BSY microcapsule carrier that could be used as a delivery system in medical applications. Soluble extracts have different emulsifying performances: some have a good performance when applying low energy (hand shaking), while others needed high energy (Ultraturrax) to have emulsifying capacity. Production of films was successful with soluble extracts, using solvent casting method, glycerol (plasticizer), genipin (crosslinker) and sepiolite (filler). The delivery system interacts with immune receptors accessed by glucan microarrays and by recognition in immune cells, using human dendritic cells.

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[SUS 11]

Development of food packaging biomaterials using brewer's spent yeast polysaccharides

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Brewer's spent yeast (BSY) is a main by-product of the brewing industry, being a source of valuable compounds, like glucans and mannoproteins. These are reported to have promising applications[1] and can be solubilized using extraction techniques producing extracts with different compositions which rule their applications. This work aims to evaluate the production of biomaterials with potential application as food packaging, using such extracts.
Production of films was successful with hot water and alkaline BSY extracts, using the solvent casting method (drying at 25-35 °C during 16-24 h), glycerol (plasticizer), genipin (crosslinker) and sepiolite (filler). Hot water extract produced a film using 20 mg sample/cm^2, 25 % (w/w) glycerol and 0.05 % (w/v) genipin, obtained by heating the solution (95 °C during 30 min) for homogenization. Film was opaque and had a dark blue colour. A wettability test indicated a hydrophilic nature since the water contact angle (WCA) was 49°. Another film produced used a 0.1M KOH alkaline extract (20 mg sample/cm^2, 50% (w/w) glycerol, 0.05% (w/v) genipin). Film was transparent, had a brownish tint, and the wettability displayed a WCA of 87°, indicating a less hygroscopic behaviour. Mechanical tests performed revealed a Young's Modulus (YM) of 67.3 MPa, a tensile strength (TS) of 2.3 MPa, and an elongation percentage (E%) of 24, hence the film was fragile and needs mechanical improvement. Final film was produced using a 4M KOH alkaline extract (20 mg sample/cm^2, 50% (w/w) glycerol, 0.05% (w/v) genipin and 1.5% (w/v) sepiolite). WCA was 86º and mechanical tests revealed a YM of 336 MPa, a TS of 8 MPa and a E% of 5, being a rigid and resistant film.
Results show that films may be produced using different aqueous extracts, allowing production of different films that can meet requirements for a specific food packaging application.

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[SUS 12]

Impact of Microwave Assisted Extraction of *Pinus pinaster* Bark under different extraction conditions

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Pinus pinaster bark is an important source of natural polyphenolic compounds and the reuse of these forest residues will play a central role in this purpose[1,2].
The aim of this study was the extraction of phenolic compounds from pine bark (Pinus pinaster Aiton subsp. Atlantica) by Microwave Assisted Extraction (MAE), using water, ethanol and ethanol:water (50:50 v/v).
Pine bark was initially dried at 40 °C for 72 hours and ground to a particle size of 200-850 µm. The MAE extraction conditions were 1600 W at 90 and 110 °C and extraction times of 20 and 30 minutes. The extraction yield ($g_{dry\ extract}/g_{dry\ bark}$), total phenolic content[3], condensed tannins [4] and anti-radical DPPH capacity[5,6] and ABTS assays[6,7] were studied.
Regarding the results, the ethanolic and hydroethanolic extracts at 110 ºC and at the extraction time of 30 min presented the most satisfactory results in all parameters, except for the condensed tannins content. The highest extraction yield was 9.9±0.0 and 9.5±0.1% (w/w) at 30 min with ethanol and hydroethanolic solvents, respectively. The greater content of polyphenols was obtained with ethanol (67.0±1.6 mgGAE/g) followed by the hydroethanolic mixture (62.9±2.5 mgGAE/g).
Regarding condensed tannins, higher concentrations were found in the ethanolic extracts at 90 °C, the extraction time showing no effect (190.7±7.8 and 189.2±1.3%, for 30 and 20 min, respectively).
The antioxidant activity (DPPH) was higher at 110 °C. The hydroethanolic samples showing the highest values in both time extractions (412.1±11.2 to 384.6±3.6 $\mu mol_{trolox}/g$, respectively), but these differences were not significant. Regarding the ABTS assay, no differences were found between solvents or extraction times.
With the MAE extraction technique at 110 ºC, it is possible to obtain pine bark extracts with high levels of antioxidant properties, using either ethanol or hydroethanolic solvents. However our aim is to achieve the maximum of antioxidant properties using the less ethanol proportion as possible, therefore other concentrations of ethanol:water mixtures will be tested in further experiments.

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[SUS 13]

Wild edible rooftops: rustic plants from green roofs provide for an alternative food source

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Building rooftops tend to have a different microclimate from that found at the ground level as there is greater exposure to radiation and wind, more so in climates with dry, hot summers.
Sturdy wild plants, found spontaneously in nature, although also possible to be cultivated, may be an interesting solution to use in green roofs (GR), increasing agricultural production in urban environments under such climates.
The Rroofood project aims at the evaluation of traditionally used wild edible plants, from the central and southern areas of Portugal, for urban farming in GR of the Lisbon area. Another goal of this project is to contribute to plant species preservation and to the development of innovative food products, while helping to find enhanced sustainable solutions for urban agriculture in GR.
Wild edible plants [1] were commonly used in ancient times in traditional dishes, but are currently little known. Recently, the use of these plants has deserved special attention, of chefs, being today a trend already associated with haute cuisine. Its regular domestic use could be revitalized, contributing to increase food diversification, based on an improved use of resources (plants and water).
In order to evaluate the cultivation potential of these plants, an experimental green roof was installed at Instituto Superior de Agronomia campus and adopted as a planting site. A preliminary trial involved plants of the following genera: *Amaranthus*, *Beta*, *Cakile*, *Chenopodium*, *Chrysanthemum*, *Nigella*, *Papaver*, *Petroselinum*, *Rumex*, *Scolymus*, *Tragopogon*, and *Viola*. Approximately two thirds of the species used successfully developed and showed crop yield potential. The results are so far encouraging but further work will allow us to verify and expand the study hypotheses.

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[SUS 14]

Valorisation of broccoli by-products as source of immunostimulatory pectic polysaccharides

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Broccoli are a source of bioactive compounds, including glucosinolates, pigments, phenolic compounds and polysaccharides. Moreover, broccoli by-products account for 45% of the broccoli heads used by frozen-food industry, sharing their nutritional value and bioactive compounds [1,2]. Therefore, the extraction of these bioactive compounds is a promising strategy for broccoli by-products valorisation as food ingredients.
In this study, pectic polysaccharides were obtained from broccoli by-products after preparation of alcohol insoluble residue (AIR), boiling water extraction, and 80% ethanol precipitation (BB_Et80). Their immunostimulatory activity was evaluated by in vitro incubation with BALB/c splenocytes and flow cytometry. BB_Et80 were further fractionated and treated with enzymes to establish structure-function relationships.
BB_Et80 represented 8% dry weight of broccoli by-products and had 66% of sugars, mainly uronic acids, Ara, Glc, and Gal, characteristic of pectic polysaccharides. BB_Et80 induced the expression of the early activation marker CD69 on the surface of B lymphocytes, in a concentration-dependent manner. Higher Ara:Gal ratio were the main contributors to the immunostimulatory activity, independently of molecular weight (12-400 kDa), charge fractionation, and removal of terminal Ara residues.
These results show that broccoli heads and broccoli by-products can be valorised as source of pectic polysaccharides with potential as functional food ingredients to improve immune function and promote health by nutrition.

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[SUS 15]

Evaluation of the antioxidant properties of pears from different Portuguese varieties: edible part *versus* by-products

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Over 23 tonnes of pears (fruits from genus *Pyrus*) are produced every year [1] and only about 75% is edible, therefore a huge amount of pears' by-products are produced every year. Pears have a composition of great interest to satisfy human needs, especially regarding micronutrients (which can be different among varieties). Furthermore, the antioxidant capacity of these fruits, specially due to the phenolic compounds, is responsible for a lower risk of several diseases [2].
The aim of this study was to carry out the comparative quantification of the total phenolic compounds, including total flavonoids, in the edible part, peel and seeds of six different national varieties of pears (Bela-Feia, Torres Novas, Carapinheira, Carapinheira Roxa, Lambe-os-Dedos and Amorim). Moreover, the antioxidant capacity of these extracts was evaluated by DPPH radical scavenging and β-carotene bleaching assays.
The results indicate that by-products are the samples that present higher TPC and TFC. However it is interesting to observe that in some varieties, the highest amount is found in peels and in other varieties, is found in seeds. The by-products showed better DPPH radical inhibition percentage (up to 25%) than the edible part (less than 10%) in all the studied varieties.
Pear by-products showed an overall better result regarding antioxidant capacity, TFC and TPC, evidencing their potential as sources of valuable antioxidant compounds. In further studies, we intend to identify and quantify the individual phenolic compounds that most contribute for the antioxidant properties of these fruits.

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[SUS 16]

Apples from Alcobaça region (Portugal): comparison of the antioxidant properties of the edible part and by-products

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Apples are one of the most produced and consumed fruits worldwide. They are a source of phenolic compounds [1], which are believed to be responsible for human health benefits such as antioxidant and anti-hypertensive activities [2]. Currently the by-products of these fruits are not used in full, therefore, it is important to carry out their characterization, namely regarding their antioxidant properties, in order to find suitable applications and avoid their waste.
The objective of this work was the determination of antioxidant properties of both by-products (peels and seeds) and edible part of five different Portuguese apple varieties (Pardo Lindo, Pêro de Borbela, Noiva, Pêro Coimbra and Repinau), produced in Alcobaça region. The antioxidant properties were evaluated through antioxidant capacity tests (DPPH radical scavenging and β-carotene bleaching [3]) and total flavonoids and total phenolic content assays. The by-products showed better DPPH radical inhibition percentage (up to 30%) than the edible part in all the varieties, as well as higher total flavonoids and phenolics content. For all the studied varieties by-products presented higher values than the corresponding edible part. Among by-products, peels presented the best results in all assays.
These results indicate that apple´s by-products have a great potential to be used as a valuable source of antioxidant compounds, which can be further used, for instance, by food, cosmetics, food packaging or food supplements industries.

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[SUS 17]

Cardoon (*Cynara cardunculus* L.): different multipurpose food industry applications

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Cynara cardunculus L. (Asteraceae) is a multipurpose crop native to the Mediterranean area, commonly called cardoon. It comprises three botanical varieties: the globe artichoke (var. scolymus (L.) Fiori), the cultivated cardoon (var. altilis DC.), and the wild cardoon (var. sylvestris (Lamk) Fiori). Cardoon can develop in adverse climate conditions, low inputs, high temperature, severe drought, and infertile soils as a resistant species. Yet, it can persist for several years, re-sprouting annually [1–3].
Cardoon can be used for different purposes due to its composition. For instance, the fleshy stems and the immature heads are used in Mediterranean cuisine. The flowers are used in the production of some cheeses as vegetal rennet. In traditional medicine cardoon is used due to its high content in cynarin and silymarin. Kidneys and liver malfunction, high blood cholesterol levels, diabetes, and cancer are some of the medicinal applications [1,4–6]. On the other hand, cardoon's by-products, leaves, stems and seeds, can be used to produce biomass for energy, biodiesel, seed oil, animal feed and paper pulp [1,3,5].
Cardoon's leaves bioactive properties (e.g.: antioxidant and antimicrobial activities) are due to its excellent polyphenol profile, rich in chlorogenic acid and flavonoids. As leaves represent, on average, about 60% of total cardoon waste, in the context of a circular economy it is essential to take advantage of its composition in bioactive compounds. Concerning the food industry, leaves, and leaves extracts bioactivity may represent an added value as a food additive, as an ingredient in developing a novel food, or in active food packaging to control lipid oxidation and microbial deterioration [1–3]. Hence, this study aims to address the current food applications of cardoon's leaves and their functional properties and explore new potential ones adding value to its properties.

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[SUS 18]

Production of a new fermented green tomato pulp: how yeast strains and addition of salt affect its physicochemical properties

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Annually tonnes of green tomato are destroyed after the ripe tomato harvesting process, what makes it the largest by-product of the tomato industry [1]. However, the fermentation of unripe tomato can be a sustainable solution for this wasted product, avoiding a loss of food, energy and resources that significantly contribute to climate change and loss of biodiversity [2,3].
Adding value to unripe tomato is the main goal of GreenTASTE Project. The fermentation process will allow to convert unripe tomato into to an intermediate product – fermented green tomato pulp – to be used as a raw material for the production of innovative and sustainable foods.
Green tomato pulps were fermented at 25 ºC with different yeast strains – *Pichia membranifaciens* and *Kluyveromyces marxianus* – in the absence and presence of 2% (w/w) salt. The fermentative performance of strains while fermenting unripe tomato was evaluated by cell viability, pH and soluble solids measurements and sugar consumption and metabolites production by HPLC. The final fermented product was also characterized in terms of colour, texture (TPA) and rheological behaviour (SAOS).
Pulps fermented by *K. marxianus* showed higher content of soluble solids, citric and malic acid; lower firmness and degree of internal structuring; and darker colour than pulps fermented by *P. membranifaciens*, although the fermentation by *K. marxianus* in the presence of salt, produced a pulp with a stronger internal structure system than the same paste fermented without salt. Furthermore, the addition of salt decreased firmness values and improved the growth ability of both strains in tomato pulp.
These results reveal that it is possible to produce an intermediate product from unripe tomato, with different physicochemical characteristics according to the fermentative yeast strain and salt concentration. Using this strategy, food industry can produce green tomato pulps with distinct physicochemical attributes, in line with their needs and consumer trends.

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[SUS 19]

Valorise foods and Improve Competitiveness through Emerging Technologies applied to food by-products within the circular economy framework-ValICET

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The Mediterranean Basin is an agri-ecological diversity hotspot with an extraordinary variety of raw materials that allow the agri-food industry to offer a very large number of traditional food products, but large amounts of wastes are produced whose disposal represents a critical hurdle to sustainability, affecting negatively economic, environmental, and social development.

Thus, in agreement with the Goals 2 and 12 of Agenda 2030 to ensure "Zero hunger" and "Sustainable consumption and production patterns", respectively [1], the ValICET project, funded by PRIMA (Section 2), and framed in the Thematic Area 3-Agrofood chain (Topic 2.3.1 (RIA5) New optimized models of Agri-food supply chain systems offering fair price for consumers and reasonable profit share for producers) [2] will focus on the development of novel strategies for the valorisation of wastes and by-products from post-farming processes of Mediterranean agri-food systems and the design of eco-friendly processes (Pulsed electric fields, PEF; High pressure homogenization, HPH; supercritical waster extraction, SWE) to recover high-added value bioactives to be reintroduced in the agri-food chain as food ingredients/additives within the circular economy framework.

ValICET project aims at developing innovative and sustainable processing solutions capable of converting agri-food residues into sustainable natural high-added value compounds to be reincorporated into the food supply chain, to improve functionality as well as to extend the shelf-life of traditional Mediterranean food products within the framework of the circular economy. An ICT solution will be deployed in order to improve the access to market of local smallholders and improve their direct contact with the different market players

ValICET addresses these challenges by an integrated interdisciplinary approach, achieved with a team including research institutions with complementary expertise, three SMEs and one large enterprise (LE) specialized in the development of food machinery and food manufacture, from five countries of the Mediterranean basin (Spain, Italy, France, Tunisia and Portugal).

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[SUS 20]

Evaluation of antioxidant activity of strawberry agro-industrial by-products and pulps

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The post-harvest processing of strawberries generates by-products, such as the inedible parts and non-marketable portion of the fruits, which are costly to disposal but can be valued. This study aimed to measure the antioxidant capacity of extracts of strawberry calyx, in order to evaluate the possibility to incorporated them into pulps to avoid browning, without compromising taste. Preliminary tests indicated that extracts with ethanol at 90% gave higher yield in antioxidants, and then extracts of strawberry calyx, fresh and pasteurized pulp were prepared in triplicate. The different extracts were used to determine the total phenolic content by the Folin–Ciocalteu method [1], the total flavonoids content according to the aluminum chloride method [1], the in-vitro antioxidant activity by the DPPH* radical scavenging method [1] and β-carotene bleaching assay [1]. Calyx extracts possessed the highest content of total phenolics (13.70 mg GAE/g) and flavonoids (11.98 mg ECE/g). Their antioxidant activity (29.23 mg Trolox/g of extract) is more than four times higher than pasteurized and fresh pulp (respectively 8.40 and 6.03 mg Trolox/g of extract). The pasteurization process potentiates the antioxidant activity, whereas reduced total phenolics and flavonoids. Due to its antioxidant potential, calyx dry extracts were incorporated in fresh pulp, using concentrations of 0.1% and 0.2%. Samples with and without extract were kept at room temperature and at accelerated conditions for 60 minutes. The incorporation of calyx extract didn't show any visible alterations in the color of pulp and a bitter taste was noticed with the higher concentration. In conclusion, this study highlights the impact of the bioactive potential obtained from strawberry agro-industrial by-products at a low cost, however further studies are needed to find the most adequate concentration that allows the equilibrium between the antioxidant activity and the impact of the taste perceived by the consumers.

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[SUS 21]

Antimicrobial properties of extracts obtained from plums, cherries and dates by-products

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Nowadays, increasing interest in the valorization of food by-products aims to improve the food's industry sustainability (1). In this study, we evaluated the extracts of fruit and their by-products regarding their antimicrobial properties. In this line, fruits and their by-products, including several varieties of plums (*Prunus domestica* L.) from Portugal, cherries (*Prunus avium* L.) from Portugal and Italy and dates (*Phoenix dactylifera* L.) from Tunisia, were tested. Some samples were freeze-dried and other were used in their fresh form.
In order to evaluate the antimicrobial activity of the extracts an antimicrobial screening test using disks diffusion methodology was performed against strains of Gram (+) and Gram (-) bacteria (including *Escherichia coli*, *Listeria inocua*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and strains of yeasts (*Candida spp.*). The diameter of the microbial inhibition was measured after 24 and 48 h. Interestingly, almost all the by-products presented higher antimicrobial inhibition than the corresponding edible parts. Moreover, dates presented antimicrobial activity against the tested strains of yeasts (2). Also, the results indicate that lyophilization might play a key role in obtaining higher antimicrobial performance.
These by-products have potential as a source of natural antimicrobials to be used in the pharmaceutical and cosmetics industry or incorporated in packaging to produce active food packaging.

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[SUS 22]

Valorization of food by-products: antioxidant properties of extracts obtained from plums, cherries and dates by-products from Portugal, Italy and Tunisia

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One of the main trends in food production is to be environmentally sustainable. In this regard, food by-products can be used to extract valuable natural compounds (e.g. antioxidants, antimicrobials, colorants) that could be used as new products or raw materials in different sectors such as food, pharmaceutical, cosmetics or packaging industries.
In this study, we produced food grade extracts from fruits and fruits by-products and further evaluated their antioxidant properties. The selected fruits were: plums (Prunus domestica L.) from Portugal, cherries (Prunus avium L.) from Portugal and Italy and dates ((Phoenix dactylifera L.)) from Tunisia, in the freeze-dried or fresh forms. The extracts were assessed for their antioxidant capacity (AC) through two different assays: the DPPH radical scavenging activity and the β-carotene bleaching assay. Their total phenolics (TP) content was evaluated by using the Folin-Ciocalteu method and their flavonoids content (FC) by using the aluminium chloride method. Interestingly, almost all the by-products of fruits obtained higher AC and TP and TF content in comparison with their edible parts.
In general, from the selected samples, date seeds extracts presented the highest antioxidant capacity. Also, the results indicate that lyophilization might play a key role in obtaining higher antioxidant performance. The extracts obtained from the tested by-products have potential as a source of natural antioxidants to be used directly as food additives. Moreover, they can also be incorporated in polymers to produce active food packaging, which aims to enable a controlled release of the bioactive compounds to the packaged foods in order to delay the deterioration at foods surface and to increase food shelf life.

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[SUS 23]

Determination of mycotoxins for the valorization of pistachio shells

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Pistachio (Pistacia vera L.) is a high-value nut highly consumed worldwide. Consequently, a great amount of shell is produced and become waste. Pistachio shell is rich in bioactive compounds with antioxidant and antifungal properties because it protects the kernel from fungal contamination [1,2]. This by-product has the potential to be included in a sustainable processing solution and reincorporated into the food supply chain [3]. However, pistachio is one of the treenuts with a higher risk of mycotoxins' contamination, especially by aflatoxins classified as proven carcinogenic to humans (IARC Group I) [4].
This study developed an analytical procedure for the determination of mycotoxins in pistachios based on Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method followed by Ultra-High Performance Liquid Chromatography combined with Time-of-Flight Mass Spectrometry (UHPLC–ToF- MS). The method was applied in pistachios shells to evaluate their safety for reuse, aiming for the circular economy.
The method was validated for the determination of eight mycotoxins (aflatoxins – AFB1, AFB2, AFG1 and AFG2; ochratoxin A; zearalenone; toxins T2 and HT-2). The validation of this method showed good recovery, repeatability, and inter-day precision in agreement with criteria established by Commission Regulation EC No. 401/2006 for mycotoxins analysis. The limits of detection for aflatoxins ranged from
0.125 to 0.25 µg/kg, considerably lower than the maximum levels in nuts regulated by the EU (AFs: 4 µg/kg).
The optimized analytical method is reliable, accurate and its low detection limit allows to evaluate the compliance of nuts with EU maximum permitted levels of mycotoxins. To evaluate the applicability of the optimized method, this was applied to eleven pistachios shells samples acquired in Portugal and in two of them aflatoxin B2 was detected, but at low concentrations (0.53 µg/kg and 0.56 µg/kg). The analysed pistachios shells were considered safe, concerning mycotoxins contamination, so, they have a possible application for use, e.g., to be used to prepare active extracts to prepare active food packaging.

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[SUS 24]

Development of biobased and thermoplastic 3D printing filaments derived from potato and coffee roasting industries by-products

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Potato industry generates by-products with biomolecules of interest for the development of thermoplastic biobased materials, namely starch-rich slurries. Starch, due to its thermoplastic properties, is a potential candidate for the development of biobased 3D printing filaments. However, the hydrophilicity and brittleness of thermoplastic starch-based materials still compromise their extrusion as 3D printing filaments. On the other hand, coffee roasting industry originates coffee silverskin (CS) as by-product, a lignocellulosic thin tegument of the coffee beans outer layer with potential to improve the physicochemical and mechanical performance of starch-based materials [1]. In this work, the influence of CS on optical, melt tenacity, wettability, and mechanical properties, as well as on the extrudability and 3D printability of thermoplastic starch-based formulations was studied. CS gave rise to brown thermoplastic starch-based formulations that, after hot-pressing, allowed to obtain transparent films with increased rigidity and water tolerance, when compared to the neat thermoplastic starch-based materials. The thermoplastic starch/CS-based formulations were efficiently extruded as 3D printing filaments with a homogeneous diameter of 1.75 mm. Furthermore, the developed starch/CS-based filaments allowed to produce 3D objects, giving place to an ecological strategy that encourages a circular economy by adding value to agrifood by-products recovered from two different industries.

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[SUS 25]

Lipid oxidation inhibition through active polylactic acid films with rosemary and green tea extracts

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More and more, consumers are demanding natural products with longer shelf-life. Extracts from rosemary (*Rosmarinus officinalis* L.) and green tea (*Camellia sinensis* L.) have high antimicrobial and antioxidant activities[1; 2]. These extracts can be incorporated into polymers produced from renewable sources, to produce active biopackaging. This concept intends to positively interact with packaged food, to inhibit the natural degradation of food, maintaining the quality of the packaged food and increasing its shelf-life [3].
The antioxidant activity of food grade extracts from rosemary and green tea was evaluated and the total content in phenolic compounds and flavonoids was determined. The extracts were incorporated in polylactic acid (PLA) in different concentrations to produce active PLA-based films. Almond was packaged with the new active packaging and stored at different temperatures and evaluated at different periods. The lipid oxidation of the almond was monitored by the thiobarbituric acid reactive substance (TBARS) assay and the peroxide value.
The rosemary extract showed higher antioxidant capacity and higher content in phenolic compounds than the green tea extract. Regarding the lipid oxidation assays, the almond packaged with PLA incorporated with 2 and 4% of rosemary extract showed lower malonaldehyde (through TBARS assay) and peroxides value at the end of 30, 45 and 60 days of storage at room temperature and 40 ºC (accelerated assay).

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[SUS 26]

PLASTICOLIGHT – Renewable lightweight fillers for plastics

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PLASTICOLIGHT is an ongoing co-promotion project between company and the University of Aveiro, supported by Portugal2020 program, focused on the development of renewable and lightweight fillers for plastics. Under a circular economy strategy, non-value agrifood byproducts, such as eggshells, potato washing slurries, and apple pomace have been used as source of molecules of interest for the development of lightweight fillers, namely calcium carbonate ($CaCO_3$), starch, and pectin, respectively. The feasibility of incorporating each developed filler into polypropylene (PP)- and polystyrene (PS)- based materials as well as their processability have been studied at both laboratorial and pilot scales.

Up to now, $CaCO_3$/starch and $CaCO_3$/pectin conjugates showed lower density than the non-renewable reference $CaCO_3$ used by Isolago. Each one of these formulations revealed compatibility with PP- and PS-based formulations and, depending on the dosage used, they allow to tune the chromatic properties, rigidity, and density of PP- and PS-based materials. Moreover, $CaCO_3$/starch and $CaCO_3$/pectin fillers did not compromise the injection molding and extrusion ability of PP- and PS-based formulations. Thus, PLASTICOLIGHT have promoted a sustainable development of renewable and lightweight fillers for plastics, creating the opportunity for adding value to various agrifood industry byproducts.

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LUPIN AND CHICKPEA: NUTRITIVE BEVERAGES AS SUSTAINABLE ALTERNATIVES TO MILK

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There is a high demand for milk substitutes other than soy beverages. Reasons for this can be found on matters ranging from health to sustainability [1,2,3,4]. However, plant-based offers, in the European market, are essentially poor in protein content (less than 1.5% against the 3.5% in milk). If one dares to change, a possible solution could be the use of pulses with high protein content. Although the beany flavor may occur, this can be easily mitigated or overcome by current processing technology, which also enhances digestibility and beverage nutritional quality.
This study compares results, before and after in vitro digestion of two beverages produced from lupin and chickpea. Lupinus albus L. and Cicer arietinum L. dry seeds were used to produce beverages with 10% (w/v) in water. After processing, beverages were submitted to static in vitro digestion [5] and analysed. Lupin and chickpea beverages and respective digesta were submitted to antigelatinolytic bioactivity analysis, to evaluate their anti-carcinogenic potential.
Chickpea-based beverages showed a protein content around 3.6% (w/v) and lupin beverage 4.7% (w/v). In vitro digestion caused a decrease on protein content as expected. Phytic acid levels were also reduced and there was no impact on protein digestibility, neither on the digestive enzymes during digestion. The existing low phytic acid contents on all digesta did not interfere in mineral composition, as divalent cations showed bioaccessibility after it. The lupin digesta evidenced significant higher contents in Ca, Mg, P, Mn, and S when compared to chickpea.
Results suggest that pulse beverages are good sources of protein, and there was evidence of protein hydrolysis by in vitro digestion and bioavailability of minerals. Both phytic acid and lectins did not inhibited digestive enzymes. In addition, besides being highly digestible, lupin and chickpea beverage showed anti-inflammatory and anti-carcinogenic potential [6].

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FROM natural ingredients TO sustainable food products

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The BIOMA Project – "Bioeconomy integrated solutions for the mobilization of the Agro-food market" intends to move entities in the agri-food value chain, to more competitive and sustainable levels, promoting strategies and an environment that enhance the adoption of integrated Bioeconomics solutions. To achieve this goal, the project seeks to respond to the problem of food waste by increasing the shelf life of products and proposes the valorisation of agri-food by-products and residues through the development and implementation of different methods for extracting active substances at the industrial level, given their value in the agri-food chain as new functional ingredients.
Some plants with recognized bioactive properties were selected to obtain natural ingredients (extracts and essential oils) and evaluate their preservative capacity. These natural ingredients will be incorporated directly or after stabilization in the internal coating of films and packaging for whole or packaged sliced fruit and peeled potatoes.
Furthermore, this project intends to recover valuable compounds from agri-food by-products generated by companies operating in the industry of minimally processed fruit and vegetables and olive oil production, to be incorporated in new value-added foods, as fruit juices, to assign them functional properties.
The final task will be the demonstration in real production environments as well as the assessment of the economic impact of applied products\technologies, which are key factors for the adoption of the natural ingredients by the industry. The use of the selected natural ingredients by the industry, also implies its inclusion in the list of authorized food additives, so a dossier will be prepared to request European Food Safety Authority (EFSA) approval.
This work is being developed in collaboration with the Portuguese Agri-food industry.

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Traceability in Food Value Chains

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Consumers and official authorities increasingly demand to be informed about the origin of the food on the market, along with its preservation conditions throughout the value chain. This requirement demands keeping track of all production activities, but also storage and transportation activities, throughout each product's value chain. These include activities that are relevant from a product production perspective, such as agricultural or aquacultural production, capturing wild fish, livestock farming, product packaging, product transformation (beef or fish processing in canneries, etc.), from which consumers are interested in the origin of natural products and their type of production, but also in the mixed origins of transformed products. And, activities that are relevant from a preservation perspective, such as storing and transporting at the right temperature, transporting from which place to what other place, so that the travelled distance and the associated carbon footprint may be assessed.
This type of information demands the use of traceability platforms, which must exist outside each company in a value chain, but must be accessible by all the companies and the final consumers. Each company must be able to add information to the platform, and everyone must be able to consult the traceability information, given a product code (e.g., the product lot number), all the way back to its initial production activity.
These types of platforms, for food traceability, may be based on different technologies and several examples already exist, for fish and fish products traceability [1, 2], products with a certified origin [3], wine supply chain traceability [4], agricultural products traceability [5], coffee value chain traceability [6], and many others.
Traceability systems register different relevant attributes for each kind of products, monitoring their quality, carbon footprint, health conditions, allowing informed decisions by final consumers, when buying the products, and enabling product lots' recall by authorities when detecting deficient health conditions.

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