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XX EUROFOODCHEM CONGRESS

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Congress organized under the auspices of the Food Chemistry Division of the European Chemical Society (FCD-EuChemS) and the Portuguese Chemical Society (SPQ).

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BICAFÉ

CASTELBEL PORTO

Porto, 17-19 June 2019

Scientific Program

17th June	
8:30-9:00	Registration
9:00-9:30	Opening Ceremony
9:30-10:10	**P1. Livia Sarkadi** "Peter Czedik-Eysenberg Lecture". The role of food chemistry in the development of food science. History and future challenge"
10:15-11:00	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Safety Room 3 – Food Sustainability
11:00-11:45	Coffee break / posters session
11:45-12:45	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Safety Room 3 – Food Processing
12:45-13:15	**K1. Nicoletta Pellegrini** "Food design and low-calorie intake"
13:15-14:30	Lunch break
14:30-15:10	**P2. Isabel Ferreira** "Functionalizing food with natural bioactive ingredients"
15:15-16:30	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Composition and Authenticity Room 3 – Food Sustainability
16:30-17:15	Coffee break and poster session
17:15-18:30	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Composition and Authenticity Room 3 – Food Processing
18:30-19:00	**K2. Lilia Ahrné** "Customised dairy products by gentle processing"
19:30	Welcome reception

18th June	
9:00-9:40	**P3. Luciano Navarini** "New trends in Coffee Research: a Chemical Perspective"
9:45-10:45	**Oral presentations** Room 1 – Food Composition and Authenticity Room 2 – Food Processing Room 3 – Food Packaging
10:45-11:30	Coffee break / posters session
11:30-12:30	Room 1 – Food Composition and Authenticity Room 2 – Food Processing Room 3 – Food Packaging
12:30-13:00	**K3 Saskia Van Ruth** Smelling fraud: sniffing spices"
13:00-14:30	Lunch break
14:00-14:25	Vendor seminar - WATERS
14:30-15:10	**P4. Jesus Simal Gandara** "What to do to fit our food system for the future?"
15:15-16:30	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Composition and Authenticity Room 3 – Food Safety
16:30-17:15	Coffee break and poster session
17:15-18:00	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Processing Room 3 – Food Safety
18:00-18:30	**K4. Yildirim Selçuk** "Active food packaging"
20:00	Congress dinner

19th June	
9:00-9:40	**P5. Gaud Dervilly-Pinel** "An ever-increased confidence in chemical food safety driven by latest analytical innovation"
9:45-10:45	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Composition and Authenticity Room 3 – Food Processing
10:45-11:30	Coffee break / posters session
11:30-12:30	**Oral presentations** Room 1 – Functional Foods Room 2 – Food Safety Room 3 – Food Processing
12:30-13:00	**K5. Ricardo Calado** "The geographic origin of seafood - Why should we care about traceability?"
13:00-14:30	Lunch break
14:30-15:10	**P6. Barbara Burlingame** "Achieving sustainable diets: the fundamental role of the food chemist"
15:10-15:40	**K6. Richard FitzGerald** "Marine protein-derived biofunctional peptides"
15:40	Closing ceremony
16:10	Farewell party

Detailed Program

17th June	
8:30-9:00	Registration
9:00-9:30	Opening Ceremony
Plenary session 1 – Chairperson: Marco Arlorio	
9:30-10:10	**Livia Sarkadi** - "Peter Czedik-Eysenberg Lecture". The role of food chemistry in the development of food science. History and future challenge"
Room 1 – Functional Foods – Chairperson: Tanja Cirkovic Velickovic	
10:15-10:30	Yan Wang - Prenylated flavonoids derived from *Flemingia philippinensis* display potent bacterial neuraminidase inhibition activities
10:30-10:45	Filipa Mandim - Influence of the harvest stage on the phenolic composition and bioactive properties of *Cynara cardunculus* L. var. *altilis* heads
10:45-11:00	Laura Mateo-Vivaracho - Study of functional properties of wild and cultivated edible mushroom powder
Room 2 – Food Safety – Chairperson: Vieno Piironen	
10:15-10:30	Sonia Losada-Barreiro - The antioxidant efficiency in O/W emulsions can be controlled by modulating antioxidant interfacial concentrations
10:30-10:45	Maria S. Silva Lopes - Mycotoxin content of *Salicornia* L. in Portugal
10:45-11:00	Caroline Douny - Development of an analytical method for the simultaneous measurement of 10 biogenic amines in meat. Application to Beninese grilled pork samples.
Room 3 – Food Sustainability – Chairperson: Victor de Freitas	
10:15-10:30	Rúbia Corrêa - Chemical composition and bioactivities of Juçara fruit bio-residues, a promising source of valuable molecules
10:30-10:45	Elisabete Coelho - Antimicrobial potential of essential oils from agro-industrial by-products as possible feed ingredients
10:45-11:00	Tassadit Benhammouche - Enhancing Proteins extraction from *Moringa Oleifera* leaves: From conventional methods to a fully integrate process
11:00-11:45	***Coffee break / posters session***
Room 1 – Functional Foods – Chairperson: José Baptista	
11:45-12:00	Franks Kamgang Nzekoue - Development of a new functional dairy product enriched in phytosterols: the importance of food chemistry
12:0-12:15	Elisabete Gonçalves - Administration of Castanea sativa flowers extract in Wistar rats
12:15-12:30	Paulina Opyd- Comparative effects of dietary hempseeds and hempseed oil on liver functions and lipid metabolism in genetically obese rats

12:30-12:45	Maria Rita Martins - Acorn Flour as bioactive compounds source in gluten free bread
Room 2 – Food Safety – Chairperson: Reto Battaglia	
11:45-12:00	Zuzana Ciesarová - Health promoting foods with sea buckthorn: more benefits, less acrylamide
12:0-12:15	Rebeca Cruz- -Is it safe to eat seafood? A case study of flame-retardants
12:15-12:30	Iolanda Nicolau-Lapeña - Selecting alternatives to chlorine for strawberry sanitation while maintaining nutritional and physicochemical quality
12:30-12:45	Lieselot Hemeryck - Investigation of diet-related DNA adduct formation by means of DNA adductomics
Room 3 – Food Sustainability – Chairperson: Susana Cardoso	
11:45-12:00	Kandi Sridhar - Kinetics modeling and effect of drying temperature on new commercial grape 'Kyoho' skin: Evaluation for functional and antioxidant properties
12:0-12:15	Franziska Hanschen - Stability of glucosinolate hydrolysis products and the identification of novel compounds in foods
12:15-12:30	Rossana Cardoso - Gamma irradiation preserves nutritional and chemical composition of *Agaricus bisporus* Portobello
12:30-12:45	Thi-Van-Linh Nguyen - Effects of Drying Conditions in Low-temperature Microwave-assisted Drying on Bioactive Compounds and Antioxidant Activity of Dehydrated Bitter Melon Slices (Momordica charantia L.)
Keynote 1 – Chairperson: Fernando Ramos	
12:45-13:15	**Nicoletta Pellegrini** - Food design and low-calorie intake
13:15-14:30	*Lunch break*
Plenary session 2 – Chairperson: Tanja Dcirkovic Velickovic	
14:30-15:10	**Isabel CFR Ferreira** - Functionalizing food with natural bioactive ingredients
Room 1 – Functional Foods – Chairperson: Nadia Mulinacci	
15:15–15:30	Bartosz Fotschki - The effect of diets supplemented with hemp and poppy seed oils on the development of obesity-related disorders in Zucker rats
15:30-15:45	Oludemi Taofiq - Enhanced extraction of ergosterol from *Pleurotus ostreatus* using response surface methodology (RSM)
15:45-16:00	Tuba Esatbeyoglu - Biological activities of stilbenoids in vitro
16:00-16:15	Giovanni Caprioli - Simultaneous quantification of 30 different bioactive compounds including polyphenols in spent coffee ground and coffee silverskin by HPLC-MS/MS triple quadrupole
Room 2 – Food Composition – Chairperson: Roberto Larcher	
15:15–15:30	Antonio Salatino - How diverse is Brazilian propolis?

15:30-15:45	Iris Tauber - Flavour analysis of an old Austrian apple variety at different ripening stages
15:45-16:00	Fernando Tateo - IRMS characterization of the saffron water-soluble fraction for the discrimination of the origin.
16:00-16:15	Carmen Gonzalez Sotelo - SEA-TRACES – Sustainable Seafood Production using Authenticity and Traceability tools
16:15-16:30	Christoph Walkner - Food authentication by rare earth element labelling and detection using solution based and laser ablation ICP-MS
Room 3 – Food Sustainability – Chairperson: Daniel Alberto Wunderlin	
15:15–15:30	Ana Rita Silva - Agrocybe cylindracea bio-residues: a sustainable source of ergosterol-rich bioactive extracts
15:30-15:45	Vera Barbosa - Tailored farmed fish iodine and selenium fortification with naturally enriched diets: gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) as case studies
15:45-16:00	Steve Huysman - At-line boar taint classification by means of Rapid Evaporative Ionisation Mass Spectrometry (REIMS)
16:00-16:15	Ana Luísa Fernandes - Anthocyanins Thermostability Modulation Through the Fortification with Pectic Polysaccharides Extracts
16:15-16:30	Filipa Pimentel - Simulated gastrointestinal digestion increases the antioxidant activity of *Porphyra dioica*
16:30-16:45	***Coffee break and poster session***
Room 1 – Functional Foods – Chairperson: Nicolas Sommerer	
17:15-17:30	Małgorzata Starowicz - Influence of heat treatment on biological compounds profile and antioxidant activity of herbs and spices and cookies with their contribution
17:30-17:45	Ecem Evrim Çelik - Determination of the Interactions between Bound and Free Antioxidants Naturally Occurring in Foods
17:45-18:00	Bianca Albuquerque - Composition in anthocyanins and bioactive properties of jabuticaba bioresidues
18:00:18:15	Vaida Kitryte - Multistep fractionation of blackberry (*Rubus fruticosus* L.) pomace into high value functional ingredients
18:15-18:30	Carlos Gomes - Valorisation of a Portuguese endemic species as a potential functional food: *Thymus carnosus* Boiss.
Room 2 – Food Composition and Authenticity – Chairperson: Sauro Vittori	
17:15-17:30	Helmut Mayer - Genetic variants of bovine milk proteins – “A2 milk” authentication using isoelectric focusing and PCR
17:30-17:45	Jing Zhang - Comparison of fatty acids and triglycerides profiles among big eye tuna (*Thunnusobesus*), Atlantic salmon (*Salmo salar*) and bighead carp (*Aristichthysnobilis*) heads

17:45-18:00	Daniel Wunderlin - Authenticity of Complex Foods: A Targeted Metabolomics Study of Authenticity Markers of Chía, Flax and Sesame Seeds in Bakery Products.
18:00:18:15	Hanna Manninen - Linking non-volatile taste compounds with the sensory profile of Nordic wild mushrooms
18:15-18:30	Tatiana Vilela - The Effect of Dissociating Agents on the Dispersion of a Grated Cheese – Conclusions for its Structure-Holding Interactions
Room 3 – Food Processing – Chairperson: Jorge Saraiva	
17:15-17:30	Stephanie Treibmann - Dicarbonyl Scavenging by Creatine in Food and in vivo
17:30-17:45	Maria Gabriela Lima - Shiitake mushroom (*Lentinola edodes*) spread creams
17:45-18:00	Alexandra Fatouros - Potential browning precursors within uronic acid reaction-systems
18:00:18:15	Ítala Marx - Monitoring physicochemical and sensory attributes during debittering of stoned green olives
18:15-18:30	Floriane Doudiès - Impact of low temperature on interactions and cohesiveness of casein micelles dispersions
Keynote 2 – Chairperson: Karel Cejpek	
18:30-19:00	**Lilia Ahrné** - Customised dairy products by gentle processing
19:30	*Welcome reception*

18th June

Plenary session 3 – Chairperson: Livia Simon-Sarkadi

9:00-9:40	**Luciano Navarini** - New trends in Coffee Research: a Chemical Perspective

Room 1 – Food Composition and Authenticity – Chairperson: Isabel Revilla

9:45–10:00	Cemile Yılmaz - Neuroactive Compounds in Fermented Foods
10:00-10:15	Lorenzo Cecchi - Volatile fraction by HS-SPME-GC-MS and sensory evaluation of more than 1200 Virgin Olive Oil samples: methods to support Panel Test in Virgin Olive Oil classification
10:15-10:30	Thelma Machado - Blockchain technology for the management of food sciences researches

Room 2 – Food Processing – Chairperson: Dulcineia Ferreira Wessel

9:45–10:00	Małgorzata Wronkowska - Bioaccessible of D-chiro-inositol from water biscuits formulated from common buckwheat flours fermented by lactic acid bacteria or fungi
10:00-10:15	Sandra Grebenteuch - Thermal deterioration of ω-3 and ω-6 fatty acids under food processing conditions
10:15-10:30	Michael Hellwig - Protein oxidation in food: Focus on individual structures
10:30-10:45	Maria Serrano - Effect of drying methods on the properties of mixtures of aromatic plants for gastronomy using different encapsulated agents

Room 3 – Food Packaging – Chairperson: Yildirim Selçuk

9:45–10:00	Paula Ferreira - BIOFOODPACK - Biocomposite Packaging for Active Preservation of Food: the project and the progresses
10:00-10:15	Ana Machado Silva - Innovation in food packaging: a retailer's perspective
10:15-10:30	Gianni Sagratini - GRAFOOD: 'Active GRAphene based FOOD packaging system for a modern society'
10:30-10:45	Ana Barra - Development of clay-supported graphene materials for electrical conductive food packaging applications

10:45-11:30 ***Coffee break / posters session***

Room 1 – Food Composition and Authenticity – Chairperson: Ewa Sikorska

11:30-11:45	Diletta Balli - Optimized hydrolytic methods by response surface methodology to avoid underestimation of phenols in cereals: the case of millet.
11:45-12:00	Antónia Nunes - Olive pomace: a multidimensional approach focusing zero wastes
12:00-12:15	Juhász Réka - Comparison of dietary fiber enriched cookies
12:15:12:30	Claudia Passos - Role of espresso and instant coffee melanoidins on chlorogenic acids and caffeine adsorption and potential immunomodulation effects

Room 2 – Food Processing – Chairperson: Hans-Jacob Skarpeid

11:30-11:45	Maria João Carvalho - Repitching impact on sugars and amino acids uptake by a lager yeast
11:45-12:00	Clemens Kanzler - Maillard induced color formation based on intermediates with activated methylene groups
12:00-12:15	Sónia Ferreira - Broccoli by-products as ingredients rich in bioactive compounds after microwave assisted dehydration
12:15:12:30	Andreia Silva - From maize flour to bread: changes in hydroxycinnamic acid and their derivatives after processing
Room 3 – Food Packaging – Chairperson: Paula Ferreira	
11:30-11:45	Zélia Ribeiro Alves - Exploring the zinc oxide – reduced graphene oxide as an active composite in alginate films for food packaging application
11:45-12:00	Carla Barbosa - Effect of MAP (high CO2%) on quality of fresh-cut non-climacteric vegetables in light of PCA with predictive biplots
12:00-12:15	Duarte Rego - BioFoodPack Pulsed Electric Fields: in-package application for microbial inactivation for food products
12:15:12:30	Gonçalves Idalina - Agrofood byproducts as feedstocks for active food packaging materials
Keynote 3 – Chairperson: Zuzana Ciesarova	
12:30-13:00	**Saskia Van Ruth** - Smelling fraud: sniffing spices
13:00-14:30	*Lunch break*
14:00-14:25	Vendors seminar – WATERS Euan Ross - Determination of acrylamide in processed foods by LC-MS/MS
Plenary session 4 – Chairperson: Hans-Jacob Skarpeid	
14:30-15:10	**Jesus Simal Gandara** - What to do to fit our food system for the future?
Room 1 – Functional Foods – Chairperson: Thelma B. Machado	
15:15-15:30	Cláudia Nunes - Structural analysis of *Nannochloropsis oculata* polysaccharides and its potential as functional food
15:30-15:45	Sónia Barroso - Response surface optimization of Phycobiliprotein pigments extraction from *Gracilaria gracilis* and application in pancakes
15:45-16:00	Giuseppina Crescente - Hemp Seed: an unthinkable source of bioactive compounds
16:15-16:30	Sheila Oliveira-Alves - Phenolic acids from baru nuts target cell proliferation in a 3D cell model of colorectal
Room 2 – Food Composition and Authenticity – Chairperson: Reşat Apak	
15:15-15:30	José Figueira - New analytical approach, NTME/GC-MS, as a powerful strategy for establishment of the volatomic profile, an alternative to SPME/GC-MS

15:30-15:45	Carolina Camacho - Carotenoids and colour of wild sea urchin (*Paracentrotus lividus*) gonads
15:45-16:00	Ewa Sikorska - Optical spectroscopy and chemometrics as a tool for quality and authenticity assessment of apple fruits and juices
16:00-16:15	Rafael Sprea - A comprehensive study on the nutritional, chemical and bioactive properties of lovage (Levisticum officinale W.D.J. Koch)
16:15-16:30	Marijana Ačanski -Hierarchical clustering of cold-pressed pumpkin seed oil samples from various sources
Room 3 – Food Safety – Chairperson: Giovanni Caprioli	
15:15-15:30	Bienvenida Gilbert-Lopez - Liquid chromatography/mass spectrometry and dielectric barrier discharge ionization (DBDI): a versatile tool for pesticide analysis in food
15:30-15:45	Tiziana Nardin - Glycoalkaloid profiles of herbal infusion using neutral loss - high resolution mass spectrometry
15:45-16:00	Carlos Cardoso - Algal consumption: weighing benefits against risks
16:00-16:15	Rosa Pilolli - Multidisciplinary characterization of selected wheat genotypes and in-silico risk assessment of their potential toxicity for celiac disease patients
16:15-16:30	Sarah Azinheiro - Comparative study of multiplex real-time Recombinase Polymerase Amplification and ISO 11290-1 methods for the detection of Listeria monocytogenes in dairy products

16:30-17:15 ***Coffee break and poster session***

Room 1 – Functional Foods – Chairperson: Franziska S. Hanschen	
17:15-17:30	Andre Horta - Extraction optimization of phenolic compounds from Fucus spiralis
17:30-17:45	Yibin Li - Effect of Different Encapsulating Agent Combinations on Physicochemical Properties and Stability of Microcapsules Loaded with Phenolics of Plum (Prunus salicina Lindl.)
17:45-18:00	Débora A. C. Campos - Pineapple by-products integrated valorisation towards functional foods
Room 2 – Food Processing – Chairperson: Michael Hellwig	
17:15-17:30	Custódio Lobo Roriz - Dragon fruits as an alternative source of colorants: evaluation of antibacterial activity and colouring capacity
17:30-17:45	Baoru Yang - Impact of Bioprocessing on Bioactive and Flavour Compounds of Berry Products
17:45-18:00	Ma. Michelle Lopez - Flavonoid enrichment of fresh-cut apple through osmotic dehydration
Room 3 – Food Safety – Chairperson: António Peres	
17:15-17:30	Tomas Lafarga - Potential of thermosonication as an alternative to chlorine disinfection in strawberries inoculated with *Listeria innocua*
17:30-17:45	Susana Bernardino - Use of a seaweed-enriched kefir whey in the development of edible food coatings
17:45-18:00	Kristina Kukurova - Variability of L-asparagine level in sea buckthorn berries and acrylamide formation in novel cereal products

Keynote 4 – Chairperson: Joana Amaral	
18:00-18:30	**Yildirim Selçuk** - Active food packaging
20:00	*Congress dinner*

19th June

Plenary session 5 – Chairperson: Michael Murkovic

9:00-9:40	**Gaud Dervilly-Pinel** - An ever-increased confidence in chemical food safety driven by latest analytical innovation

Room 1 – Functional Foods – Chairperson: José Câmara

9:45–10:00	Mafalda A.M.M. Silva - Functional features of two varieties of *Cucumis melo* L.: pulp and by-products
10:00-10:15	Maja Jeż - Functional properties of tomatoes in the aspect of inhibition of advanced glycation end products formation and activity of antiangiotensine and antiacetylocholinesterase
10:15-10:30	Ana Margarida Faustino - Goat Probiotic Whey Cheese: Development and Nutritional Value
10:30-10:45	Maria Cermeno - Generation and characterisation of phenolic rich extracts from brewers' spent grain with hypotensive properties

Room 2 – Food Composition and Authenticity – Chairperson: Thelma B. Machado

9:45–10:00	Diana Melo - Trends in human diets: Seeds as a source of fibre
10:00-10:15	Nicolas Sommerer - Linking cocoa polyphenol composition to chocolate quality with Average-Mass-Spectra fingerprints
10:15-10:30	Mustafa Reşat Apak - Criteria for selection of antioxidant capacity assays for food antioxidants: Evaluation of CUPRAC, FRAP, Folin-Ciocalteau, ORAC, ABTS and DPPH assays

Room 3 – Food Processing – Chairperson: Małgorzata Wronkowska

9:45–10:00	Csilla Benedek - Effect of sweetener and storage on formation of compositional and sensory properties of jams
10:00-10:15	Pedro António Fernandes - Flavan-3-ols in apple pomace: role of their interactions with arabinans
10:15-10:30	Tanja Seppälä - Quality of protein concentrates isolated by two different methods from Baltic herring (*Clupea harengus membras*) and roach (*Rutilus rutilus*)
10:30-10:45	Susana Soares - Interaction of human salivary proteins with food polyphenols

10:45-11:30 ***Coffee break / posters session***

Room 1 – Functional Foods – Chairperson: Manuel António Coimbra

11:30-11:45	Priscilla Figueira - In vitro investigation of the health promoting benefits from different food matrices: From extraction to characterization and in vitro evaluation
11:45-12:00	Henghui Zhang - Preparation and Purification of Antioxidative Peptides Generated from Enzymatic Hydrolysates of Perilla Seed Meal
12:00-12:15	Ana Rita Circuncisão - In vitro bioaccessibility and bioavailability of minerals and pigments from macroalgae-fortified frankfurters

Room 2 – Food Safety – Chairperson: Vaida Kitryte	
11:30-11:45	Ly Tuan-Kiet - Matrix effect in multi-pesticide residues analysis: the complexity in tea commodities
11:45-12:00	João Reboleira -I.FILM: Multifunctional films for application in active and smart packages
12:00-12:15	Luiza Kijewska - Depletion of methylene blue from muscles of rainbow trout (*Oncorhynchus mykiss*)
Room 3 – Food Processing – Chairperson: Michael Murkovic	
11:30-11:45	Antia Gonzalez Pereira - Extraction of phenolic compounds by high hydrostatic pressure from eight edible algae species from the North-West coast of Spain: Process modelling and optimization
11:45-12:00	Elisabete Alexandre - Pomegranate peel extraction optimization and characterization and their inclusion in carrot juice improving their safety and quality
Keynote 5 – Chairperson: Baoru Yang	
12:30-13:00	**Ricardo Calado** - The geographic origin of seafood - Why should we care about traceability?
13:00-14:30	Lunch break
Plenary session 6 – Chairperson: Vieno Piironen	
14:30-15:10	**Barbara Burlingame** - Achieving sustainable diets: the fundamental role of the food chemist
Keynote 6 – Chairperson: Małgorzata Starowicz	
15:10-15:40	**Keynote 6** Richard FitzGerald - Marine protein-derived biofunctional peptides
15:40	*Closing ceremony and awards*
16:15	*Farewell party*

PLENARY SESSIONS & KEYNOTES

Peter Czedik-Eysenberg lecture

The role of food chemistry in the development of food science History and future challenge

Livia Simon Sarkadi
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The modern history of food chemistry in Europe started in the middle of 1970' when the Working Party on Food Chemistry (later Division) established within the Federation of European Chemical Societies (now European Chemical Societies, EuChemS) by Peter Czedik-Eysenberg. The main aim was to bring together food chemists across Europe and to promote the development of the food science.

The Food Chemistry Division took leading position in the past and I hope to follow this trend for the future to offer scientific forum for food scientists to discuss current research results and plan multidisciplinary projects for collaboration.

Following the sudden death of Prof. Peter Czedik-Eysenberg (1929–2001), as a mark of respect and deep affection, the Division established the Peter Czedik-Eysenberg Memorial Lecture to deliver by an internationally recognised food chemist at the opening of the Division's flagship EuroFoodChem conferences. I am extremely honoured to be selected for this prestigious lecture of the XX EuroFoodChem conference in Porto, Portugal.

Peter Czedik-Eysenberg was a great champion and promoter of young scientist especially of women at every level in science. I certainly benefitted from his advice and encouragement.

In my presentation, I would first like to describe the development of food chemistry in Europe since the formation of the Food Chemistry Division, to consider the current situation and, finally, to look into the future.

Functionalizing food with natural bioactive ingredients

Isabel C.F.R. Ferreira*
Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Portugal
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Natural sources such as plants and mushrooms have been extensively studied for their nutritional properties and are considered important components of a balanced diet. Beyond nutritional features, these matrices are also rich sources of bioactive molecules that exert valuable health benefits. Some of these compounds have proven their efficacy as antioxidant and antimicrobial agents, and others have also found application in food colouring processes, which makes them excellent food preservatives and colorants. From this point of view, the reformulation of foodstuff through the introduction of these bioactive extracts with different functionalities (bioactive, preservative, and colouring molecules), can be considered a functionalization strategy, allowing food properties improvement.

Several compounds extracted from plants and mushrooms were applied in food matrices. For instance, phenolic acids (e.g. rosmarinic acid), flavonoids (e.g. quercetin derivatives), and ellagitannins (e.g. sanguiin H-10 and lambertianin) from mushrooms, wild strawberry, rosemary, mountain sandwort, and flowers of *silva brava* demonstrated bioactive properties when introduced in gelatin, yogurt, and cottage cheese [e.g. 1]. On the other hand, betalains (e.g. gomphrenin II, gomphrenin III, isogomphrenin II, and isogomphrenin III) and anthocyanins (e.g. cyanidin, delphinidin, and malvidin derivatives) obtained from purple globe amaranth, rose, dahlia, centaurea, strawberry-tree, roselle, and blueberry have proved bioactive and colouring properties when incorporated in ice-cream, yogurt, and waffles [e.g. 2]. Moreover, strawberry-tree, basil, lemon balm, sweet chestnut flowers, fennel, and German chamomile revealed to be great sources of preserving molecules with antioxidant and antimicrobial activity, such as flavonoids (e.g. catechin, and quercetin and luteolin derivatives), phenolic acids (e.g. rosmarinic, chicoric, lithospermic, caffeic, and caffeoylquinic acids), and hydrolysable tannins (e.g. trigalloyl-HHDP-glucoside), which were tested in loaf bread, cupcakes, yogurt, cheese, and cottage cheese [e.g. 3].

The results obtained allowed to conclude that natural extracts from plants and mushrooms can be used for food functionalization.

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New trends in Coffee Research: a Chemical Perspective

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Coffee, in addition to be one of the most consumed beverages in the world, has always been one of the most studied. The multiplicity of cultivation, harvesting and post-harvest methods and the related economic relevance in many Countries, the complexity of its aroma and the methods to generate and extract it and the effects on health are some of the reasons that have made this product the topic of many projects and the object of study for numerous research groups. In the last decade, however, interest has even grown in areas that have gradually become more important, such as sustainability and circular economy under the pressure of climate change, authenticity, product traceability and food safety to guarantee consumers more and more. and the role played by biologically active compounds other than caffeine and nutrigenomics in light of the several positive effects on health thanks to moderate consumption. In this framework chemistry provides a valuable contribution that is declined in new methods of analysis and characterization, new processes and insights into knowledge in the different elements of the long chain of quality from the plant to the cup. The present work aims to offer an overview of some of the aspects mentioned above highlighting the areas where it would be important to pay more attention in the future.

What to do to fit our food system for the future?

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There is a big shortfall between:

- the amount of food we produce today, and
- the amount needed to feed everyone in 2050.

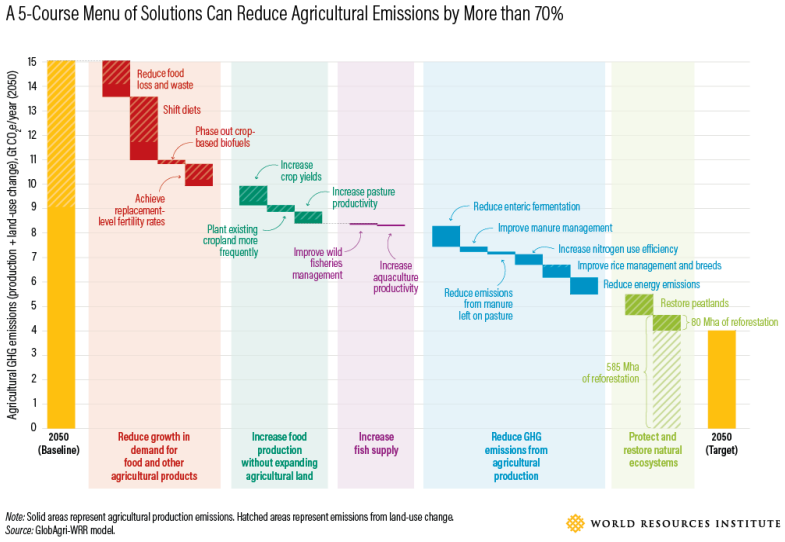
Feeding 10.000 million people sustainably by 2050, then, requires closing three gaps:
1.-A 56 percent food gap between crop calories produced in 2010 and those needed in 2050 under usual growth;
2.-A 593 million-hectare land gap (an area nearly twice the size of India) between global agricultural land area in 2010 and expected agricultural expansion by 2050; and
3.-An 11-gigaton GHG (Green-House Gases) mitigation gap between expected agricultural emissions in 2050 and the target level needed to hold global warming below 2oC (3.6°F), the level necessary for preventing the worst climate impacts.

WRI (World Resources Institute) has identified 22 solutions that need to be simultaneously applied to close these gaps. The solutions are organized into a five-course menu (see Figure below):

(1) reduce growth in demand for food and other agricultural products;
(2) increase food production without expanding agricultural land;
(3) protect and restore natural ecosystems;
(4) increase fish supply; and
(5) reduce GHG emissions from agricultural production

The relative importance of each solution varies from country to country.

In conclusion, the challenge is much harder than people realize. These menu items are not optional, but necessary, to close the food, land and GHG mitigation gaps. All five courses can close the gaps, while delivering co-benefits for farmers, society and human health. It will require major changes to how we produce and consume food:
1.-consuming less resource-intensive animal-based foods,
2.-cutting greenhouse gas (GHG) emissions from agriculture, and
3.-stopping conversion of forest to agricultural land.



An ever-increased confidence in chemical food safety driven by latest analytical innovations

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To increase consumers' confidence in their diet, official food control systems rely on laboratories with measurement technologies that are adapted to the growing demands of the field. They face the uninterrupted extension of the spectrum of chemicals to which humans are exposed but also the need to characterize these molecules at ever lower concentrations. The methods developed to answer such analytical challenges are characterized by a very high selectivity and a strong sensitivity for the targeted approaches but are also able to generate information without a priori in capacity to point out emerging dangers or to reveal markers of exposure or effect. A review of analytical methods - essentially based on mass spectrometry - will be proposed, including some historical background, the present state of the art and perspectives. Several examples of application will be presented in the light of progress made in the tracking of banned substances in cattle rearing or in the measurement of historical and more emerging environmental contaminants. Multidimensional mass spectrometry, high or very high resolution, will be discussed in the context. The interest of ion mobility will be debated with regard to the selectivity provided in complex matrices. Alternative sample introduction strategies to gas or liquid chromatography such as Ambient Mass Spectrometry (ASAP, REIMS, DART), SFC or GC-APCI will be illustrated. The advent of non-targeted approaches relying on mass spectrometry metabolomics workflows will be commented and informed by applications revealing biomarkers of effect in response to the use of anabolic substances in cattle rearing. All benefits in terms of time analysis, specificity/selectivity or sensitivity of signals will be widely discussed together with limitations/pitfalls associated with these cutting edge technologies.

Achieving sustainable diets -- the fundamental role of the food chemist

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'Sustainable diets' has proven to be a useful concept for simultaneously addressing both human and environmental health. Underpinning sustainable diets is food composition and the chemists undertaking the analyses of nutrients, bioactive non-nutrients and contaminants in food.
After considerable consultation and debate, sustainable diets were defined as those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. They are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources. (Source: FAO, 2010).
Making the case for sustainable diets' fundamental role in food security and nutrition seems intuitive, but this is not sufficient when policies, programmes and interventions need to be evidence based. Food composition science, i.e., the realm of the food chemist, is the humble research specialty that provides the most compelling evidence.
In this presentation, the uses and benefits of food composition data will be illustrated with research findings and case studies with a focus on four elements in the definition of sustainable diets: biodiversity, ecosystems, fair, and safe.
Examples include:

- Biodiversity: Pacific Island banana biodiversity and vitamin A
- Ecosystems: Mongolia and n-3 fatty acids
- Fair: Biotechnology vs biodiversity and the case of orange-fleshed sweet potato

Food design and low-calorie intake

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Food and nutrient digestibility has been a mantra that has been accompanying us ever since we arrived on the earth. However, today, the situation has completely changed. Especially in the countries where people follow a Western diet, the major concern should no longer be a high digestibility of food and how to gain energy from it but rather how to reduce and control the energy intake, since the present scenario describes a world that is becoming fatter and fatter. The highly processed foods are contributing to the increase in obesity not only because of their nutrient composition but also because of their high digestibility. This high digestibility is partly linked to the lack of natural structure of foods severely processed. Viceverse, plant-based foods, which are not severely processed, largely retain their natural structure. Mounting evidence suggested that preserving the natural structure of plant foods may have beneficial effects on health, e.g. by increasing the feeling of satiety and providing a slower release of glucose and fatty acids. When cellular integrity is retained, macronutrients are “encapsulated” within cell walls. This encapsulation hinders macronutrients to digestive enzymes and bile acids reducing their digestibility and absorption in the upper intestine. Thus, intact legumes, nuts and cereals contain also low-digestible starch, proteins and lipids. These concepts should be kept in mind when we redesign foods with the aim of reducing the energy release e.g. of gluten-free bread that is rich in carbohydrates with high digestibility. Including legumes to gluten-free bread is widely appreciated particularly to counteract the lack of nutrients, but it can be effective also to reduce the starch digestibility when their cell wall integrity is retained.

Gentle processing - potential and challenges

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Gentle processing intends to preserve the fresh-like properties of foods, limiting the damage on the nutritional and sensory properties, while at the same time providing the final product with a shelf life sufficient for distribution and storage. Thermal and non-thermal technologies are commercially available, or emerging, offering new possibilities for gentle processing. They are governed by different physical mechanisms and consequently their impact on microorganisms, food structure and individual components differs significantly. Knowledge and understanding of the effects of gentle techniques on food can be explored to customize products and ingredients with naturally enhanced functionality. In this presentation will be reviewed the potential and challenges of gentle processing using traditional, novel or combination of technologies.

Smelling fraud: sniffing spices

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Consumers are frequently confronted with news regarding food fraud incidents. Although it may seem that food fraud incidents have only occurred in recent years, food fraud is as old as mankind. Nowadays, with the exposure of global food fraud scandals and its economic implications for the food industry, general quality assurance systems are giving more attention to food fraud management by recognising the need to ensure product authenticity through the requirements of risk analysis and control plans. Despite all efforts in science and industry, a lot of questions remain. What drives these fraudsters? What are the risk factors? Who are likely to become victims? In which way would detection help to prevent food fraud? How widely is fraud spread? Which developments are on the horizon? These questions will be discussed in the presentation, using spices as an example.

Active Packaging for Food: Opportunities, Technologies and Challenges

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One of the main functions of the food packaging is to maintain food quality and safety from the production until consumption by preventing any unwanted chemical and biological changes. The protective role of the packaging is primarily passive, acting as a barrier between the food, the atmosphere surrounding the food, and the external environment. Over recent decades there is an increasing trend to natural high-quality foods, which are non-processed or minimally processed, do not contain preservatives, but offer an acceptable shelf-life. In response, the protective function of packaging has been refined and improved leading to the development of new packaging technologies such as active packaging (AP) [1]. AP systems are designed to "deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food." AP materials are thereby "intended to extend the shelf-life or to maintain or improve the condition of packaged food. [2]" While predominantly researchers focus on the extension of the shelf life through use of AP technologies, AP offers much more benefits. Use of AP may contribute to the decrease of food lost especially for perishable products with short shelf lives, reduction or removal of food preservatives such as antioxidants, antimicrobials from food formulations, or enable to use particular type of packaging material such as a more sustainable alternative. It may also simplify the food processing by being an additional hurdle for the food preservation or enable to develop a new product at all which would not be possible without AP applications.

Active packaging systems can be divided into active scavenging systems (absorbers) and active-releasing systems (emitters). Whereas the former remove undesired compounds from the food or its environment, for example, moisture, carbon dioxide, oxygen, ethylene, or odor, the latter add compounds to the packaged food or into the headspace, such as antimicrobial compounds, carbon dioxide, antioxidants, flavors, ethylene, or ethanol [3]. Among the active packaging systems, oxygen scavengers have the highest potential as they can show several benefits such as prevention of discoloration, mold growth, browning and rancidity as well as retention of nutritional value. Moisture scavengers may help to extend the shelf life, decrease the condensation in the packaging and can have a positive impact on the appearance by reducing the browning or discoloration.

Despite the several studies on AP, only a very few of the potential solutions have been able to reach the market. In addition to the socio-economic and legislative factors, technical challenges are the most important factor for the failure to reach to the market. Major technological challenges are availability, quality, stability and consistency of the active compounds, successful integration of the active compounds into the packaging material, interaction of active compound and the food components and control and activation of the AP technologies. Additionally, lacking of fast and reliable quality control systems, which should be available to all the stakeholders and impact of active components on the environment as well as on recycling of the packaging, hinders the successful introduction of such AP technologies into the market.

In this paper, AP technologies and their potential benefits for food applications will be presented and the challenges for successful introduction of such technologies into the market will be discussed.

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The geographic origin of seafood - Why should we care about traceability?

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The unprecedented level of market globalization, along with a growing awareness of consumers on food safety issues, makes food traceability an urgent and challenging task. Traceability is defined by the EU as “the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution”. However, the traceability of seafood has often lagged behind when compared to the efforts to enforce traceability from land-based food items, namely meat. While the fight against the mislabeling of seafood has been the main target of policy makers and governmental agencies, the reliability of claims on the geographic origin of seafood made by different players along the supply chain is now starting to deserve attention. Being able to verify the geographic origin of seafood is paramount to impair fraudulent practices associated with the illegal capture of seafood, which may pose a threat to the sustainability of wild stocks, as well as consumers safety. Being able to pin-point the geographic origin of seafood is mandatory if authorities want to efficiently enforce closed seasons of target fisheries or impose effective bans from specific regions due to food safety risks. Molecular, biochemical and geochemical tools are currently available to verify the claims made by the supply chain on the geographic origin of seafood. The present talk will showcase the use of these tools to trace the geographic origin of different types of seafood, such as seabass, clams and goose barnacles.

Marine Protein-Derived Biofunctional Peptides

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The marine proteome (including fish, shellfish, micro- and macroalgae) contains a large array of peptides with the potential to beneficially modulate key biomarkers of health and disease. The literature provides extensive examples of marine sourced peptides with potential antidiabetic, antioxidative, hypotensive, mineral binding, etc, properties [1-5]. These peptides can be released during the manufacture of protein hydrolysates, during fermentation, during gastrointestinal transit or by a combination of all these events. Various *in vitro*, *in situ* and *in vivo* approaches have been employed to demonstrate and characterise the bioactive potential of complex peptide mixtures, enriched fractions of peptides and indeed individual peptide sequences. The identification and ultimate validation of biofunctional marine-derived peptides is associated with many challenges. These include: the choice of starting material; the method of peptide release; peptide bioavailability and efficacy; biopeptide stability; interactive effects with other peptides, food components and possibly drugs; and finally peptide identification and understanding of their action mechanism. A detailed understanding of the mechanism of action can allow an *in silico* aided targeted generation and subsequent enrichment of hydrolysates having high bioactive potential. Ultimately, a detailed knowledge of those peptide structural features which are essential for bioactive potency is necessary. Therefore, examples of the identification and characterization of hypotensive, antidiabetic and antioxidative peptides from marine protein substrates will be outlined. A detailed characterization and *in vivo* validation of biofunctional peptides/peptide preparations ultimately facilitates approval by regulatory authorities and eventual uptake by consumers.

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VENDOR SEMINAR

Determination of acrylamide in processed foods by LC-MS/MS

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Acrylamide is a well-known contaminant formed at high temperatures, during the production of starch containing foods. Acrylamide's toxicological properties have been extensively studied and is classified as a group 2A carcinogen by the International Agency for Research on Cancer (IARC).

Acrylamide recently hit the headlines internationally in March 2018, when a judge in California ruled acrylamide fell under the State's Proposition 65 labeling requirements. At a similar time, in April 2018 the EU Regulation 2017/2158 was enacted, requiring that food business operators wishing to trade in the EU manage acrylamide within their food safety management systems.

In this talk we will introduce a new method using LC-MS/MS that has been developed, to provide a rapid, cost-effective approach for quantifying acrylamide in coffee.

Single laboratory method validation was completed using a selection of store purchased processed food products and reference coffee and potato chips samples (purchased from the FAPAS proficiency scheme). The performance of the method was assessed in terms of linearity, precision and trueness, using the criteria of Commission Regulation (EU) 2017/2158.

In house verification of the modified QuEChERS approach showed excellent sensitivity and LC-MS/MS performance for the detection, identification, and quantitation of acrylamide in a selection of processed food samples. Validation of the method demonstrated excellent performance in terms of linearity, accuracy, precision and repeatability, in accordance with the criteria outlined in Commission Regulation (EU) 2017/2158.

ORAL COMMUNICATIONS

Food composition and Authenticity

How diverse is Brazilian propolis?

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Propolis is a honey bee product containing beeswax and resin obtained from plants, in addition to pollen and other minor constituents. Propolis is an important product for the biology of honeybees, including defense against microbial infection and enhancement of the hive immunity [1] A wide diversity of biological activities has been ascribed to propolis. All known activities of propolis are exerted by substances derived from the plant sources of resin. In Brazil, propolis is produced by Africanized *Apis mellifera* (Apidae). Each propolis type has a characteristic chemical profile. Several types of Brazilian propolis are associated with biomes or ecosystems. For example, Brazilian green propolis characteristically contains prenylated phenylpropanoids and is derived from vegetative buds of *Baccharis dracunculifolia* (Asteraceae), a species from "cerrados" widespread in southeast and central Brazil [2]. Instead, Brazilian red propolis contains isoflavonoids and is produced with exudates of *Dalbergia ecastaphyllum* (Leguminosae, Fabaceae) in mangroves from northeast Brazil [3]. A green propolis is produced in the "17Ptinga", a semiarid biome exclusive of Brazil. This propolis type derives from buds of *Mimosa tenuiflora* (Leguminosae, Mimosaceae) [4].

Despite the plant mega diversity of the country, relatively few types of Brazilian propolis have been reported. Several constraints account for the seemingly low number of plant sources of Brazilian propolis. Among them, stand out: a) the requisite that the plant source of resin must be able to provide exudates or soft tissues amenable to be licked or chewed by the fragile honeybee mandibles; b) the plant material should contain substances with antimicrobial activity; c) the biologically active substances must be neither unpalatable nor toxic to the honeybees. A small percent of plant species complies with all characteristics required to fulfill the role of sources of propolis resin.

Acknowledgements: the authors thank CNPq and FAPESP for provision of funds used in their research about Brazilian propolis.

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Flavour analysis of an old Austrian apple variety at different ripening stages

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With more than 80 %, Styria is Austria's main producer of apples grown in plantations as well as in meadow orchards. The majority of apples harvested, are cultivated in plantations, with the most common varieties being Golden Delicious, Gala or Idared. Only a small amount of the apple harvest originates from meadow orchards, where traditional old Austrian apple varieties like Ilzer Rose, Herbstkalvil or Krummstiel can still be found. Many of the old apple varieties exhibit remarkable flavour characteristics, which are not yet fully described. A deeper knowledge of the flavour of old apple varieties can aide producers with the production of high quality products like juices and ciders. Unfortunately, old apple varieties are almost extinct from the market. To prevent this, there have been experiments to test a possible cultivation of old apple varieties in plantation growing. As a result, the old Austrian apple variety Ilzer Rose is now cultivated in plantations.

The aim of this work is to characterize the flavour of an old apple variety (Ilzer Rose) cultivated in plantation growing at different ripening stages in two consecutive years (2017 and 2018). The flavour components are analysed using several different techniques providing complementary information:

i. Gas chromatography-mass spectrometry GC-MS and comprehensive GC × GC-MS

ii. New techniques of sensory analysis.

For the investigation of the volatile compounds, extraction and enrichment are carried out using headspace solid-phase micro-extraction. For the identification of the volatile and odour active compounds GC-MS and comprehensive GC × GC-MS are applied. The enormous capacity regarding separation as well as sensitivity of comprehensive GC × GC-MS allows deep insight into the flavour of the old apple varieties. The complete sensory evaluation of the sliced apples after enzyme inactivation characterisation is performed by an expert panel.

The combination of these complementary techniques will hopefully allow the decoding of the flavour of selected apple varieties, broaden the understanding of apple flavour formation and prevent the extinction of the old apple varieties.

IRMS characterization of the saffron water-soluble fraction for the discrimination of the origin.

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The IRMS (isotope ratio mass spectrometry) analysis has long being applied to studies of identification or discrimination of the origin of various food matrices [(1- 5)]. On the other hand, the simplification of sample preparation methods for IRMS analysis is a real necessity, in order to consider IRMS measures as applicable to routine analysis.

For the saffron characterization, and also for the purpose of the useful correlation with other quality indices, the water-soluble fraction was taken into account by determining the IRMS values in a series of samples of declared origin from Iran and Greece.

Obtained data, correlated with those inferred from the GC-OCI (total carbon number) analysis of the triglyceride fraction, allowed to highlight clear discriminating features between the samples of two different origins. The ANOVA (Analysis of Variance) bootstrap was applied to the chemical characterization considering two different groups by using the geographical origin as criterion, and the KNN (K-Nearest Neighbors) analysis was singularly and jointly applied by accounting the analytical parameters. The Euclidean distances samples were also used to build the Box plot, and the statistical analyses were performed with the SPSS statistical software.

The paper allows to add a new valid analytical criterion to those adopted until now, and based on other measurements aimed to the discrimination of the origin of various raw materials.

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SEA-TRACES – Sustainable Seafood Production using Authenticity and Traceability tools

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Labeling and Traceability are essential to protect and valorize fishery and aquaculture products. Illegal fisheries, fraud and mislabeling are the main issues representing a serious risk to the existence of this important economic activity for many European regions, especially those of the Atlantic.

SEA-TRACES is an Interreg project aiming to demonstrate, through case studies in different regions, how innovative implementation of traceability and labeling instruments will facilitate and increase the marketing and revenues, thus acting as a driving force to inspire other companies to adopt similar strategies. Consumers and industry are meant to find agreements about how product information has to be delivered and obtained in order to maximize the information flow, SEA-TRACES will foster the exchange of views and needs between these two important actors. Appropriate analytical tools are essential for the compliance with latest labeling regulations; SEA-TRACES will develop, test and implement new tools for the verification of seafood labels. Administration, Control and Research Labs are the main actors involved in label analysis and control, involvement of these actors in newly virtual platform will allow SEA-TRACES test the feasibility of transferring results.

Some of these aspects and preliminary results of SEATRACES will be presented in this talk.

Food authentication by rare earth element labelling and detection using solution based and laser ablation ICP-MS

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In order to meet the increasing customer demand for local food products, various methods for verification of food origin by means of trace element fingerprinting have been developed in the last years. However, these methods rely on a close relationship between plants or animals and the local geology, where trace elements are absorbed from the soil into the plant or by feed intake into the animal. For products from conventional agriculture, where animals are fed commercially available complete feed, and plants are grown on substrates of different origin, this relationship is usually not given and other methods for food authentication are required.

In an alternative approach, foodstuffs produced in a certain region, by a specific producer or under certain conditions can be safeguarded against imitation by chemical labelling. The objective of the present study was to develop a method for labelling food products of both plant and animal origin by selective enrichment of two rare earth elements (REE) in the soil for plants or in the feed for animals. Therefore, a distinctive REE pattern is artificially introduced which can be detected in food products by suitable analytical techniques, and allows distinction from unlabelled products of other origin.

Labelling experiments were conducted with tomatoes, chickens, lambs and dairy goats. REE labels in food products were detected using a combination of high pressure acid digestion and solution nebulisation ICP-MS (SN-ICP-MS). Alternatively, laser ablation ICP-MS (LA-ICP-MS) was applied, allowing direct analysis of samples of mainly inorganic composition such as egg shells and bones. In addition, for samples such as meat and milk, considerable enrichment of REE can be achieved by a very simple sample preparation procedure consisting of dry ashing and pressing pellets. Therefore, the lower sensitivity of LA-ICP-MS is compensated, while matrix effects caused by enrichment of matrix elements such as Ca are less severe compared to SN-ICP-MS.

Results show that already after a short time, REE from spiked feed or soil are incorporated into food products such as tomatoes, eggs, chicken and lamb meat and goat milk, allowing discrimination from unlabelled products by the analytical methods employed. At the same time, REE contents in all products remained low enough to maintain safety for the consumer.

Genetic variants of bovine milk proteins – "A2 milk" authentication using isoelectric focusing and PCR

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Milk protein polymorphism has received great interest within the dairy industry because of possible relationship to milk production traits, milk composition (e.g., milk protein content, concentration of single milk proteins), and technological properties (e.g., heat stability, renneting and cheesemaking properties) of milk [1, 2]. In most studies, the effect of single casein loci rather than the whole set of linked casein genes has been considered, so recent studies paid attention to the effect of composite milk protein phenotypes. However, many relationships are still controversial, and several findings suggest correlations might be different among the breeds. Interestingly, the distribution of milk protein genotypes was shown to vary considerably between the dairy cattle breeds [3]. Moreover, it has been reported that the frequencies of some milk protein loci have changed during the last years, probably unintentionally or due to breeding programs (e.g. κ-Cn B, β-Cn A^2; β-Lg B) that have already been performed in several countries (e.g., Austria, Italy, Switzerland, New Zealand) [4]. β-Casomorphins (BCMs) are opioid-like peptides (chain length of 4–11 amino acids) released from β-casein of bovine or human milk during technological processes and/or enzymatic digestion in the intestine. And according the "A1/A2 hypothesis", milk can be classified into two types, i.e., A1 "like" milk having a histidine at position 67 that determines the enzymatic cleavage of the peptide bond releasing β-casomorphin-7 (β-CM-7), and A2 "like" milk that does not cleave due to the presence of a proline at this position [5, 6]. A number of studies have suggested that bovine β-CM-7 can act as a causative agent of cardiovascular disease, type 1 diabetes, sudden infant death syndrome, autism and schizophrenia [6, 7]. However, it appears highly unlikely that evolutional (natural) selection retained a harmful product as the main nutritional component for mammalian infants, and most data indicate the positive action of milk-derived peptides [8]. Finally, a scientific report of EFSA concluded that a cause-effect relationship between the oral intake of β-CM-7 or related peptides and aetiology or course of any suggested non-communicable diseases cannot be established [9].

As such dairy products (e.g., A2 milk and yoghurt) are on the market and are advertised offensively as "risk-free" A2 milk or "indigenous" milk, the authentication of these high-prized alternative foods is crucial to avoid misleading consumers by adulteration. Therefore, the aim of this study was to establish reliable analytical methods for the detection of genetic variants using isoelectric focusing (IEF) of milk proteins, and appropriate polymerase chain reaction (PCR) techniques.

The simultaneous separation of genetic variants of caseins and whey proteins using IEF was successfully applied for the phenotyping of dairy cows. Moreover, allele-specific PCR was performed to identify the β-casein alleles A^1, A^2, B, C and I. In addition, κ-Cn and β-Lg B genotypes were determined by PCR-RFLP and/or AS-PCR using specific primer-pairs and appropriate restriction enzymes. DNA extraction techniques from various sources (i.e., milk, yoghurt, blood, saliva, hair, and sperm) were optimized. Authenticity of "A2 milk" products in Austria was monitored by analysing both individual cows and retailed yoghurt and liquid milk products.

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Comparison of fatty acids and triglycerides profiles among big eye tuna (*Thunnus obesus*), Atlantic salmon (*Salmo salar*) and bighead carp (*Aristichthys nobilis*) heads

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Big eye tuna (Thunnus obesus), Atlantic salmon (Salmo salar) and bighead carp (Aristichthys nobilis) are three representative marine and fresh water fishes. In this study, their total lipids, lipid classes and fatty acids profiles were analyzed, and the complicated triglyceride (TG) molecular species of the above three kinds of fish heads were further characterized. The results showed that TG was the major lipid in these three fish heads (60.58-86.69%), then followed by phospholipids (PL) (9.04-31.66%). Among them, big eye tuna head has the most abundant polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)with the amounts of (EPA+DHA) counting for 64.29%, 32.77% and 39.68% in total fatty acids, TG, and PL fraction, respectively. It is also worth noting that (EPA+DHA)/TFA value of bighead carp showed no significant difference with Atlantic salmon, known as a typical marine fish. Based on LC-MS/MS analysis, there were 146 TG molecules detected in big eye tuna head, 90 TG molecules detected in both in Atlantic salmon and bighead carp heads. The TG molecules that containing DHA or EPA accounted for 56.12%, 22.88%, and 5.46% of total TG molecules in these three fish heads, respectively.

According to principal component analysis (PCA), OPLS-DA (Orthogonal projection to latent structures-discriminant analysis) and the constructed heat map, the three samples could be completely separated based on their TG molecules finger prints.

Key words: Fish heads, lipid classes, DHA, triglyceride molecular species, lipid content

Authenticity of Complex Foods: A Targeted Metabolomics Study of Authenticity Markers of Chía, Flax and Sesame Seeds in Bakery Products

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Food fraud is committed with increasing frequency in the food industry and the need of tools to detect it, is a current concern for producers. [1] Chia, flax and sesame are mainly known by their oils, but also they are rich in antioxidant compounds (Polyphenols). These molecules prevent cell aging and the risk of having some diseases such as diabetes, cancer, Parkinson's and Alzheimer's. [2] Because of these positive effects, it is important to verify their presence in food, but there is a lack of knowledge regarding authenticity of these seeds. This creates the need to differentiate them through the determination of biomarkers.

The aim of this work was to generate a method to determine the presence of chia, flax and sesame seeds in bakery products using polyphenols as markers. Then, the markers found in seeds were searched in cookies made at our research facility [3] to determine which compounds resisted the processing. After that, to evaluate the usefulness of the methodology, markers found in our cookies were searched in different commercial bakery products containing the seeds (sweet cookies, crackers, bread, puff pastry dough and breadsticks).

44 polyphenols were tentatively identified and quantified in the seeds by HPLC-ESI-qTOF (MS/MS) in negative mode. [4] Multivariate statistical methods were performed and 12 compounds were proposed as novel markers. Rosmarinic acid, sesaminol dihexoside and eriodictyol hexoside were proposed for chia, sesame and flax seeds respectively, among others. All of these compounds were found in cookies with seeds added at different percentages, meaning that these compounds resisted processing and they are able to be used as markers of the seeds in complex foods.

These markers were also evaluated in commercial cookies, added with different types and percentages of seeds. We found five of the markers that were proposed in our method, and certify the presence of the seeds (what was declared in the labels).

In conclusion we were able to develop a targeted metabolomic method suitable for the determination of the presence of chia, sesame and flax seeds in bakery products.

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Linking non-volatile taste compounds with the sensory profile of Nordic wild mushrooms

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Edible mushrooms are a wide group with a vast variety of different flavors. The flavor of mushrooms results from a spectrum of different volatile and non-volatile compounds. Umami is one of the key components in the taste of mushrooms. Free amino acids, 5'-nucleotides and nucleosides were analyzed from four Nordic wild mushroom species (curry milkcaps, porcinis, chanterelles, trumpet chanterelles) using high precision liquid chromatography [1]. To our knowledge, these compounds were studied for the first time from trumpet chanterelles and curry milkcaps. Furthermore, nuclear magnetic resonance spectroscopy was used to quantify sugars, sugar alcohols and organic acids from samples of mushrooms [2] and sensory profiling with a trained panel was conducted [3]. The correlations between taste compounds and perceived taste were estimated with partial least squares regression in order to find the relationships between the sensory perception and concentrations of the compounds [2].

The free amino acid and 5'-nucleotide/nucleoside contents of species differed from each other. Umami amino acids were among five major free amino acids, while the content of umami enhancing 5'-nucleotides was low in all species. Of the organic acids, sugars and sugar alcohols, mannitol, trehalose, and malic acid were the major compounds. Based on partial least squares analysis, porcinis had the closest correlation with umami and sweetness as well as total sugars. Curry milkcaps were correlated with pungency, bitterness, astringency and metallicity and had the highest concentration of organic acids but also umami amino acids. The suppressing effect by these strong tastes and the low umami nucleotide content in curry milkcaps may explain the difference in perceived taste and measured high umami amino acid concentration. With the partial least squares model we were able to identify non-volatile compounds contributing to selected oro-sensory properties in edible mushrooms.

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The Effect of Dissociating Agents on the Dispersion of a Grated Cheese – Conclusions for its Structure-Holding Interactions

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Cheese can be described as a bi-continuous gel structure consisting of a porous protein matrix (casein) interspaced with localized domains of fat [1]. The way this matrix is formed is key, since it plays a role in the final microstructure of cheese and, consequently, its texture, flavour and overall quality [2] [3].
This work used Emmental cheese, which is a Swiss-type, semi-hard curd cheese. In these cheeses, the matrix acidification occurs after pressing of the curd, i.e., when most of the whey has already been expelled. Hence the concentration of colloidal calcium increases proportionally with the concentration of casein at drainage, leading to the formation of a highly cohesive and mineralised matrix [2].
Urea, SDS, EDTA, NaCl and NaOH solutions are commonly used as protein denaturants. In this work, we aimed to determine the contribution that each type of interaction had in cheese structure. For that, we used the different dissociating agents referred above, at different concentrations and combinations [4].

In this work, in order to determine total protein in cheese, we developed a modification of an UV absorbance method, preceded by the dissolution of the samples in NaOH [5]. The method was also adjusted to quantify protein in solutions where cheese was dispersed.
Sample solutions of dissociating agent and Emmental cheese were heated to 70 °C with constant agitation. This protocol, involving heating the cheese, pretends to approximate the conditions cheese is subjected when preparing cheese-containing food products, such as sauces or processed cheeses, among others. Afterwards, the samples were centrifuged, and the supernatant separated. Aliquots of this were diluted in NaOH 0.1 M, before measurement by UV absorbance at 280 nm.
The solubilized protein by each dissociating solution was then evaluated and compared to the total protein in cheese.

Results showed that a cooperation between hydrophobic interactions, hydrogen bonds and ionic interactions are involved in the protein structure of Emmental cheese, since combinations of urea, SDS and EDTA show a high solubility for the cheese proteins. Urea, alone, at the concentration of 6 M, was able to solubilize up to 85% of cheese proteins, while urea (6 M), SDS (2.5%) and EDTA (4 mM) combined were able to solubilize up to 92% of the cheese proteins.

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Neuroactive Compounds in Fermented Foods

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Humans have been consuming fermented foods such as bread, wine and beer for thousands of years. Why people chose these foods brings up the topic about the relationship between food and mood, which is still under investigation. Apart from nutritional components of these foods, amino acid derivatives, which have generally neuroactive properties, may play an important role in human mood and cognition. Neuroactive compounds are synthesized by microorganisms in fermented foods. Therefore, the aim of this study was to investigation of some neuroactive compounds in fermented foods. Moreover, the effects of different fermentation conditions, yeast/bacteria fermentation, and interaction between microorganisms on these compounds were highlighted. During fermentation, changes in the concentrations of neuroactive compounds clarified by linking their precursors, especially tryptophan, and metabolic pathways. This study addressed the neuroactive compounds and relevant precursors in beer, bread and wine as yeast fermented foods, and yoghurt, cheese, and kefir as lactic acid bacteria fermented foods. Melatonin, serotonin, dopamine, gamma-aminobutyric acid, kynurenine, kynurenic acid, and bioactive amines were the principal compounds investigated throughout this study. It was found that fermented foods contained these substances in a wide range and therefore, this investigation would bring new insight into the assessment of the health-promoting value of the fermented foods. Different fermentation processing such as top and bottom fermentation of beer, interactions between bacteria in yoghurt, concentrations of amino acids as precursors of neuroactive compounds were found to be effective on these compounds via this large-scale study.

Volatile fraction by HS-SPME-GC-MS and sensory evaluation of more than 1200 Virgin Olive Oil samples: methods to support Panel Test in Virgin Olive Oil classification

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Extra virgin olive oil (EVOO) is considered as the highest quality product among the edible oils thanks to its pleasant taste and smell and the health properties given by the high content of phenolic compounds. Virgin olive oils are classified as extra virgin olive oil (EVOO), virgin olive oil (VOO) or lampante virgin olive oil (LVOO), based on their chemical and sensorial characteristics [1]. To date, the official method for assessing the sensorial properties is the Panel Test, carried out by a panel of 8-12 trained tasters and a head-panel. This analysis suffers of some drawbacks due to the use of humans, as emotionality, subjectivity, low reproducibility and high costs [2]. To date it is considered increasingly necessary to have availability of robust and reliable methods for support panel test in virgin olive oil classification, only based on chemical analysis and chemometric tools [3].

Aim of this work is to propose models for supporting panel test in virgin olive oil classification through predictive systems able to correlate chemical and organoleptic properties, developed working on more than 1200 virgin olive oil samples. Furthermore, we aimed at getting further light on the molecules able to discriminate between the different categories of samples.

To this aim, we analyzed the volatile fraction (VF) of all selected virgin olive oil samples by a recently validated HS-SPME-GC-MS method [1], which allowed us quantifying up to 73 volatile organic compounds using 9 internal standard for area normalization. Sensorial characteristics of the same samples were then assessed by the Carapelli's Panel Test, acknowledged by Italian Ministry. At the same time, two oils with different fatty acid composition were stored for six months in several non-accelerated oxidative conditions and periodically analysed to better investigate the rancid defect.

Different statistical approaches have been applied in order to create predictive models aimed at discriminating between EVOO and defective samples, to support panel test in virgin olive oil classification. We considered as a key-factor working with a high number of samples in order to have very robust statistical models and with almost all the selected samples belonging to EVOOs or VOOs with only a little number of LVOOs. The capability in discriminating between samples with oxidative and microbiological defects was also evaluated. After quantifying 73 volatile organic compounds for more than 1200 virgin olive oil samples, the obtained data were analyzed together with the sensorial data using statistic tools as *t*-test and Principal Component Analysis (PCA) for reducing the dimensionality of the data, and Linear Discriminant Analysis (LDA) to find combinations of variables and finding a linear fit able to separate categories of samples.

All the proposed approaches resulted able to predict correctly the category of approx. 80% of samples. The main defect of almost all the defective samples was correctly identified. Finally, those VOCs able to better discriminate between different categories were identified and resulted the same identified in in the oil samples stored in non-accelerated oxidative conditions.

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Blockchain technology for the management of food sciences researches

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Recently there is great interest in the latest developments in the fields of big data and machine learning aimed at assisting decisions in the area of scientific research [1]. The success of scientific researches focusing on the role of diet in human health depends on an interdisciplinary approach that allows the decentralization of information among networking research groups. The use of blockchain technology offers innumerable advantages in collaborative networking, here especially addressed to functional foods. In this context, traceability of functional food research from "research group to research group" requires a complex distribution and processing system that involves everything from human resources to materials, methodologies, equipment and environment. In order to solve this problem, the advantage use of blockchain and IoT technologies to monitor and register the processes chain, as well as give each networking partner access to register all the steps of the research is proposed.
The object of discussion in the present work is the research involving functional foods (here, natural products of vegetable origin) with bioactive and/or prebiotic properties. It was designed a solution for mapping the effectiveness of research management parameters, presenting blockchain technology as a decentralized solution aimed at safety and traceability of results in the scientific process. In this way, the search for data through blockchain can be done, providing tools to the networking research groups to make validations and contributing to the safety and reliability of results.

To address this solution, the case of a promisor functional cereal (*Coix lachryma-jobi*; adlay) cultivars was taken as example. Adlay has gathered a great attention in recent years due to many health-beneficial components, including proteins, polysaccharides, coixol, and lipids [2]. If, at least in theory, the growing states and shipments of adlay are recorded in a blockchain, the record would make it easier to find out where fruits and seeds were farmed, and to which shipments they were exposed and delivered. The blockchain technology could also help companies and public health officials determine which communities might have been exposed to the products.
Mapping the efficiency of functional foods research management, six critical variables that are directly involved with the supply chain and scientific state-of-the-art were established, i.e., Field and Agricultural practices, Processing, Materials, Equipment and Environment, Methodologies, Results and Human resources. The blockchain technology helps build up a platform where information is shared, and efficiency and security rules are executed automatically. The business architecture design for mapping efficiency parameters defines the main events that will be worked with smart contracts. These smart contracts will be responsible for managing permissions, executions and ledger records using a chain code that manages the business logic introduced in blockchain and defining the accepted business contexts through Blockchain Hyperledger for all involved parties [3]. Thus, all nodes will have access to the data according to their permissions and the agents will be able to perform their research activities with the safety, transparency and traceability that Blockchain technology offers. Through the use of Hyperledger framework, researchers can create their own Blockchain in a simple, fast and without the need for much technical knowledge, because Hyperledger, through its composer, offers ready-made business models that can be specialized for more diverse purposes.

In conclusion, blockchain can support the scientific research chain, in this case, specifically addressed for mapping the efficiency of functional foods research management. This architecture can be replicated for any type of research as the benefits of the technology will be present.

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Optimized hydrolytic methods by response surface methodology to avoid underestimation of phenols in cereals: the case of millet

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Millet, which include various small grains belonging to the *Poaceae* family, can be considered as an alternative crop that exhibits great advantageous compared to other cereals; for this reason, it is called "nutritious millets" or "nutricereals". Millet is suitable for growing almost everywhere thanks to its resistance to drought, high temperature tolerance and low incidence of mycotoxin contamination [2]. Despite its advantages, this cereal is under-utilized for human nutrition in Europe to date [3]. On the contrary, in Africa and Asia, millet is classified as a subsistence crop; approximately 90% of world production is destined for human consumption. Millet possesses high levels of proteins, minerals and vitamins that confer it higher nutritional value than other cereals. Furthermore, it is rich in Slowly Digestible Starch (SDS) and Resistant Starch (RS), resulting in slow release of glucose in the blood stream and reducing postprandial glycaemic and insulinemic response. Many evidences highlighted how the consumption of this cereal, taken daily in high doses, has been associated with reduced risks of chronic diseases such as cardiovascular diseases and type-II diabetes [1]. The healthy effects of millet have also been associated with its phenolic compounds, present in free and bound forms (conjugates with sugars, fatty acids or proteins), and associated with antioxidant properties, which can be even improved after fermentation processes. To date, the methods applied for the phenolic extraction from cereals and from millet have not been compared in terms of extractive efficiency. Our goal was to define the best procedure for extracting millet phenols in order to apply this method for other cereals.

Three different batches of millet were evaluated in terms of free and bound phenols. All the quali-quantitative evaluations were performed by HPLC-DAD, ESI-MS and MS^n analysis. A total of 12 phenolic compounds were identified, mainly *C*-glycosylated flavonoid and cynnamic derivatives.
The richest batch in terms of bound phenols was selected for comparing acidic and basic hydrolyses. Our findings pointed out for the first time that methyl ferulate is one of the main component of bound phenols in millet. Response Surface Methodology (RSM) was then applied to both acidic and basic hydrolytic extractive conditions; the acidic procedure, optimized in terms of extractive time, temperature and acidic concentration, gave the best results, allowing definition of Method Operable Design Region and quantitation of the total phenols in millet samples in a single extractive step, unlike what is reported in the literature. Acidic hydrolysis gave the highest amount of total phenolic compounds (up to 174 mg/100g) while the commonly used basic hydrolysis underestimated the phenolic concentration. This optimized method resulted suitable for investigating different varieties of this recently re-discovered minor cereal, as well as for the evaluation of phenols also in other grains.

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Olive pomace: a multidimensional approach focusing zero wastes

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The olive oil production comprises three main steps: olives crushing/milling into an olive paste, malaxation, where the paste is slowly mixed, and centrifugation. The products obtained are olive oil and pomace (OP). OP is the major by-product generated in olive oil processing and it is the olive remaining pulping material after removal of the oil, consisting in water, pulp, skin, and stone. OP is a rich source of added-value bioactive compounds with reported positive effects in human health and well-being [1]. Particularly, OP phenolic compounds have been studied as preservatives and/or to improve the products nutritional profile. Furthermore, in the cosmetic field, phenolics can have particular interest in, for example, anti-ageing formulations [1]. Simultaneously, those compounds have a detrimental effect on the environment due to their phytotoxicity. Currently, OP is used for the production of edible OP oil. A dried defatted OP (extracted olive pomace - EOP) is the last remaining product of this process. Essentially, it is used as biomass for heat and energy production. However, due to the amount of phenolics, the EOP is still an environmental burden. As the olive oil production is increasing worldwide, this concern of the olive oil sector will increase.

This work aims to present a multidimensional approach for OP valorisation and answer to the economy circular concepts, focusing a zero residues strategy. For that, three main procedures are presented: 1) OP processing to obtain a functional ingredient for further incorporation in foodstuffs (Patent PCT/IB2018/060111); 2) OP secondary by-product, resultant from process 1), for the R&D of cosmetic products; and 3) EOP processing to obtain an agricultural substrate (Patent PCT/IB2017/053422).
Considering point 1) the functional ingredient was extracted physically from fresh OP obtained in an olive mill (Alfândega-da-Fé, Portugal) in the 2017/2018 season. Briefly, the obtention of the functional ingredient comprised the OP malaxation and compression (200 – 300 bar). After centrifugation (5000 rpm; 20 min), a paste still remains. Hence, its potential application as cosmetic ingredient was preliminary explored by the assessment of the total phenolics (TP) content. The TP as well as the antioxidant activity (ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl scavenging activity ($DPPH^{\bullet}$)) were assed spectrophotometrically [2]. Regarding point 3) the patented process was applied to an EOP sample obtained in Coimbra (Portugal), in April 2018. To recover the TP the sample was subjected to an aqueous extraction (1:40 m/v; 2 hour cycles; 40±5 °C; 600 rpm). Afterwards, it was filtered and the solid phase was collected and dried. Two products were obtained: an aqueous extract and the solid substrate. In both, EOP and substrate, the TP was determined [2] as well as the germination index (GI) to assess the viability for agricultural application [3].
The functional ingredient presented high content in TP (≈10 g gallic acid equivalents (GAE)/L of extract) and high antioxidant activity (97.98 mmol ferrous sulfate equivalents and 8.5 g trolox equivalents/L, assessed by FRAP and $DPPH^{\bullet}$, respectively), which reinforce its utilization in the food industry. The paste presented an inferior amount of these compounds (≈0.1 g GAE/L). For point 3), results showed that the EOP before processing has a content of ≈7 g GAE/kg of OP and a GI of 0.3% whereas the substrate (after processing) presented ≈1 g GAE/kg and a GI of 94%, meaning that's suitable for plants growth.
In conclusion, considering the patented processes described, it is possible to develop innovative and valuable products using OP: a functional ingredient for incorporation in foodstuffs and a substrate for agricultural applications. Further studies are still needed focusing the development of cosmetic products using the extracts derived from the studied processes. Hereby, zero residues can be accomplished if these processes are applied to OP.

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Comparison of dietary fiber enriched cookies

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Dietary fiber intake in European countries is below the amount recommended by nutritionists and dietetics [1]. Water soluble fibers are valuable because of their potential to inhibit both constipation and diarrhea, facilitate mineral absorption from colon and beneficially influence the microbiota in gut. Utilization of dietary fibers requires optimization of intake, because a minimal dose is required to obtain beneficial effects and overconsumption may result gastrointestinal discomfort [1]. Cookies are popular products of food industry and very suitable for precision dosage of fiber intake.

In present study three different water soluble dietary fiber: inulin, beta-glucan and xylo-oligosaccharides were used in cookies baked according to AACC 10-50D standard method [3]. Physical properties such as surface colour, texture of cookies were measured in order to compare the effect of the dietary fibers on baking quality.

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Role of espresso and instant coffee melanoidins on chlorogenic acids and caffeine adsorption and potential immunomodulation effects

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Coffee is one of the most abundant sources of high molecular weight (HMW) brown compounds known as melanoidins, which have been associated to several health beneficial properties[1]. Their content represents nearly 20% of espresso coffee total solids but, taking into account the adsorbed low molecular weight (LMW) compounds, the value represents more than half of coffee brew dry matter[2]. Because different types of brews preparation can give origin to different melanoidin compositions, in this study, instant coffee (richer in arabinogalactans) and espresso coffee (richer in galactomannans) melanoidins adsorption capacity towards chlorogenic acids (CGA) and caffeine were evaluated. Furthermore, potential synergistic or antagonicst effects between the HMW and LMW composition were evaluated on *in vitro* model assessing both immunostimulatory and anti-inflammatory potential.

Expresso and instant coffee brews were prepared and submitted independently to an ultrafiltration process using a membrane of 100 kDa cut-off. The solution was concentrated with recovery of the correspondent filtrate. The process was repeated several times and, each time, the retained solution was diluted with water and concentrated by ultrafiltration. In this way, one retentate (melanoidins rich fraction) was obtained for each coffee, as well as several filtrate fractions. The immunomodulatory potential of coffee and its relationship with coffee composition was evaluated using macrophage (Raw 264.7) cells through quantification of nitric oxide production. The anti-inflammatory potential was evaluated by incubating macrophages with the *Toll-like* receptor 4 agonist lipopolysaccharide while measuring the production of the pro-inflammatory mediator NO.

The coffee brews did not show significant macrophages activation. However, the removal of adsorbed LMW compounds from coffee brews, independently of the brew source, enhanced the formation of NO, allowing to infer immunostimulatory potential. On the other hand, the LMW material showed a potential anti-inflammatory activity. The high capacity of coffee melanoidins to adsorb LMW compounds, including chlorogenic acids and caffeine, may allow the modulation of their release along the intestinal tract, and to perspective also a health improved potential modulation of the inflammation processes *in vivo* after coffee intake during digestion.

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New analytical approach, NTME/GC-MS, as a powerful strategy for establishment of the volatomic profile, an alternative to SPME/GC-MS

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Lemon (*Citrus limonum*) is a well-known citrus fruit widely used throughout the world. Lemon varieties, and also lemons from the same variety but from different geographic regions, have different organoleptic and nutraceutical characteristics. The study of the volatile composition of lemon will allowed to differentiate lemon fruits according to geographic region.

This study was performed to gain deep insights on the volatile composition of lemon (Eureka variety) from different geographical origins. Overall, 75 VOCs, belonging to different chemical groups, namely monoterpenes, sesquiterpenes, alcohols and carbonyl compounds, were identified in the peel of targeted citrus fruits by a new analytical approach, GC-MS analysis after isolation using Needle Trap Microextraction (NTME). The new analytical approach NTME/GC-MS was submitted to optimization, namely sample amount, extraction volume, sample temperature, headspace volume and equilibration time, using an experimental design (DoE) procedure. For the Solid Phase Micro Extration (SPME) procedure, it was used the same conditions of sample amount, sample temperature and extraction time as the ones establish for NTME.

The obtained data matrices were submitted to principal component analysis (PCA) revealing that the VOM profiles were able to differentiate lemon fruits according to geographic region.
As far as we know, is the first time that this extraction technique is used in food research. The chromatographic data combined with advanced multivariate statistical analysis could be used to define potential “markers compounds” of lemons providing a useful tool for its authentication according to farming regions contributing with the compliance of current regulations about geographical protection of food. In addition, a brief comparison with the volatomic profile obtained through SPME/GC-MS strategy, shown a higher peak resolution with the NTME/GC-MS strategy. Moreover, NTME shown a higher selectivity for the volatile compounds, whereas SPME had higher selectivity for the semi-volatile compounds.

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Carotenoids and colour of wild sea urchin (*Paracentrotus lividus*) gonads

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The sea urchin *Paracentrotus lividus* found and harvested in Atlantic and Mediterranean areas, is highly valued and appreciated due to the intrinsic characteristics of its gonads (or roe). The gonad´s demand is strongly influenced by quality, being appearance, particularly colour (bright orange/yellow), the major determinant. Colour is highly depending of dietary intake and is thought to be due to carotenoid content and its precursors direct deposition or modification in the gonad tissue [1, 2]. The aim of this study was to identify the carotenoid content in both wild males and females gonads of wild *P. lividus* (Fig. 1a) caught off in Ericeira area in the peak of resource availability, i.e., before spawning (March 2018) and establish a relation to gonads colour.

Pools (at least of 4 specimens, randomly selected) from each gender were prepared. Gonad colour was measured with a colorimeter using the CIELab system. Carotenoids extraction (Fig. 1b) and HPLC-PDA analysis were conducted according a modified method [3]. The chromatograms of the separated carotenoids were detected at 450 nm and identified by comparison of their retention times and ultraviolet-visible spectra with commercial standards (Fig. 1c).

Three carotenoids were identified, and echinenone was the dominant in both genders, ranging between 30 and 60 % of total carotenoids. Lutein and β-carotene were found in lower concentrations. A few other carotenoid compounds also appeared, however due to the lack of appropriate standards these compounds were unable to be identified. Within the same gender, some variability between pools were also observed. In general, female gonads presented higher L* and a* values, nonetheless a clear link to carotenoids were not perceived. These results could be useful for the characterization and selection of highest quality gonads and a contribute to feed modulation in echinoculture.

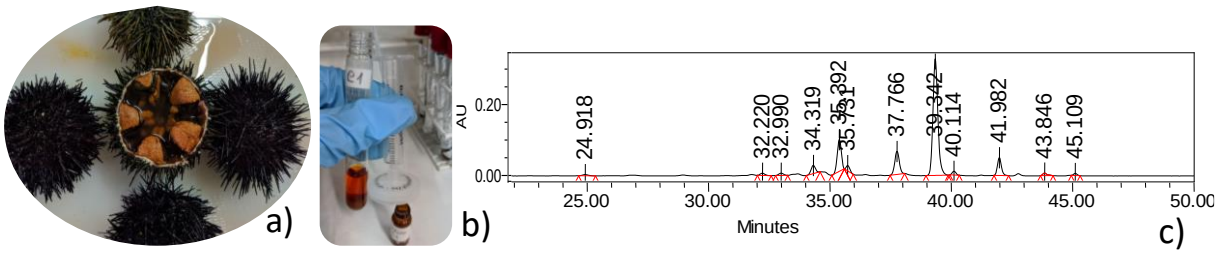

Fig.1. Specimens of sea urchin *Paracentrotus lividus* (a), carotenoids extraction (b) and a chromatogram at 450 nm (c).

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Optical spectroscopy and chemometrics as a tool for quality and authenticity assessment of apple fruits and juices

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In this presentation the application of various optical spectroscopic techniques coupled with chemometrics in assessing different aspects of apple fruits and juices quality will be demonstrated. Over the past decades, spectroscopy coupled with chemometric analysis has been shown to be a suitable tool for monitoring quality of a wide range of food products. The spectra for food products, contain information about different components of the sample matrix and their interactions. The numerous bands present in the spectra depend on the structure of the food molecular components, and their characteristics also affected by the physical properties of the sample, leading to a unique spectral fingerprints. Such fingerprints analysed using chemometric methods are used for qualitative and quantitative analysis of food [1]. The practical application of such methods relies on developing the calibration models, which describe the relationship between the spectra and particular properties of sample. Once such calibration models will be established, the property of interest could be predicted from spectra of the respective tested sample. As a result, the traditional chemical analyses could be replaced by the simpler and faster spectral measurements, Figure 1.

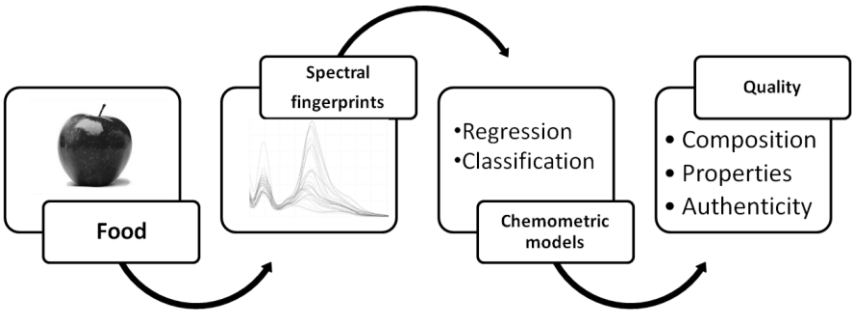

Figure 1. Application of multivariate models in food quality assessment

The aim of this study was to investigate capabilities of various optical spectroscopic techniques in quality evaluation of intact apples and apple juices. Apple is one of the most popular and widely consumed fruit. It is appreciated for its pleasant taste and high nutritional value, providing a source of phenolic compounds, vitamins, minerals, and dietary fibres. Fruit juices may provide an alternative to fresh fruit in a healthy diet. The optical spectroscopic techniques applied in this study include vibrational and electronic absorption spectroscopy in UV, Vis and NIR regions and fluorescence. The problems studied comprise determination of chemical components and properties such as soluble solid content, titratable acidity, total polyphenols content, antioxidant capacity, and evaluation of authenticity of juices. We found that the analysis of products using different spectroscopic techniques gave more comprehensive information, enhancing analytical and research capabilities.

Finally, we concluded that optical spectroscopy in association with chemometric analysis is a promising technique for the rapid screening of apple fruits and juices quality. The proposed methods have several advantages as compared to conventional analytical methods. The methods can be applied directly, on the intact samples, avoiding any pre-treatment. They are fast, simple, time- and cost- effective as well as environmentally friendly.

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A comprehensive study on the nutritional, chemical and bioactive properties of lovage (*Levisticum officinale* W.D.J. Koch)

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Plants are a considerable source of natural products and are often used by man for their aromatic, medicinal and flavouring abilities. Aromatic herbs, in particular, have been used for centuries in gastronomy but also in traditional medicine. Considering that a growing number of consumer's associate products containing natural ingredients to a higher quality, the food industry is also giving a greater importance to the possible use of plant extracts and/or essential oils as alternatives to synthetic additives in foods. Lovage (*Levisticum officinale* W.D.J. Koch) is an aromatic plant used as a condiment in several regions of Europe that is also used as an ingredient of commercial food seasonings. So far, studies on lovage mainly focused on its essential oil composition, but there is still a scarcity of information regarding its nutritional and chemical composition as well as its bioactive properties. To help start bridging this gap, and to explore alternative uses of this plant species, the present work reports a comprehensive study on lovage composition and bioactivity.

Fresh plant specimens were commercially acquired in October 2018 at Porto, Portugal. After lyophilization, the aerial parts of the plant were further analyzed. The proximate composition (moisture, fat, proteins, ash, carbohydrates and energy) was evaluated by AOAC official procedures, free sugars and tocopherols were determined using liquid chromatography coupled to a refraction index (HPLC-RI) and fluorescence (HPLC-FL) detectors, respectively. Fatty acids were determined by gas chromatography coupled to a flame ionization detector (GC-FID), organic acids by ultra-fast liquid chromatography coupled to a diode detector (UPLC-DAD) and phenolic compounds by HPLC coupled to a DAD and mass spectrometry (MS) using the electrospray ionization interface (ESI). Multi-resistant clinical bacterial strains were used to screen the antimicrobial activity of aqueous (decoction) and hydroethanolic extracts. The antioxidant activity was evaluated through five different *in vitro* assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power, inhibition of β-carotene bleaching, inhibition of lipid peroxidation in brain homogenates by thiobarbituric acid reactive substances (TBARS) and oxidative hemolysis inhibition assay (OxHLIA). The cytotoxic activity was assayed using MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) human tumor cell lines, and also a non-tumor cell line (porcine liver primary cells, PLP2).

L. officinale presented a high percentage of moisture with proteins being the predominant macronutrient. In what concerns the chemical composition of the plant, lovage showed a predominance of polyunsaturated fatty acids with alpha-linolenic acid being the major compound; alpha tocopherol stands out among the other isoforms of vitamin E; oxalic acid has a prominence among the identified organic acids, and glucose was the main free sugar present in the plant. Regarding phenolic compounds' composition, a total of 7 compounds, including phenolic acids and flavonoids, were identified and quantified, with 5-*O*-caffeoylquinic acid being the predominant one. Both extracts presented interesting antioxidant properties, and in general, were more active against Gram-negative bacteria, while only decoction extract showed cytotoxicity against a HepG2 cell line. In brief, an extensive and detailed study was performed revealing that lovage has an interesting composition from the nutritional point of view being also a source of several bioactive compounds, therefore its inclusion as a seasoning/flavoring agent in different dishes should be promoted.

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Hierarchical clustering of cold-pressed pumpkin seed oil samples from various sources

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It is evident that both the production of cold pressed pumpkin seeds oil (PSO) and its consumption have been on a constant increase in the last few years. Cold pressed PSO is a relatively new product on the Serbian market [1]. In this paper five samples of PSO were analysed using a GC/MS system. Two samples, cold-pressed oil Olinka (P1) and virgin oil Štajersko from Hungary (P2), were obtained from Department of Food Preservation Engineering at the Faculty of Technology, Novi Sad, Serbia. Three samples of cold-pressed oil: unknown cultivar (P3), Olinka (P4) and Olivija (P5), were obtained from Oilseeds Department at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. The obtained PSO samples were derivatized with 0.2 M trimethylsulfonium hydroxide solution (TMSH, Macherey-Nagel) in methanol and analyzed on a gas chromatograph (Agilent Technologies 7890). Transesterification derivatization procedure occurred in the injection port of a GC device itself, thus converting the components of oil saponifiable fraction into corresponding volatile fatty acid methyl esters (FAMEs) [2]. Eluting FAMEs were detected and identified in selected ion monitoring mode using a mass spectrometer (Agilent Technologies 5975 MSD) with NIST14 and Wiley7 mass spectra libraries. Peaks of the selected molecular ions on ion current chromatograms with the following *m/z* ratios were integrated: 268 (*cis*-9-hexadecenoic acid, methyl ester), 270 (hexadecanoic acid, methyl ester), 292 (*cis,cis,cis*-9,12,15-octadecatrienoic acid, methyl ester), 294 (*cis,cis*-9,12-octadecadienoic acid, methyl ester), 296 (*cis*-9-octadecenoic acid, methyl ester), 298 (octadecanoic acid, methyl ester), 324 (*cis*-11-eicosenoic acid, methyl ester), 326 (eicosanoic acid, methyl ester), and 354 *m/z* (docosanoic acid, methyl ester). The integrated surface areas of FAMEs were used in multivariate data analysis. Hierarchical clustering was performed applying both online platform Heatmapper [3] and PAST 3.21 program [4] and using paired group algorithm and correlation similarity measure. Data are visualized in the form of heatmap and dendrogram. By observing a heatmap it can be concluded that molecular ions of only four FAMEs contribute to differentiation between the analyzed PSO samples: 270, 294, 296, and 298 *m/z*. These were exploited for hierarchical clustering and creation of a similarity dendrogram. Dendrogram clearly shows that even with the most discriminating factors, a high level of mutual similarity between all analyzed samples of cold pressed PSO is obtained ($y > 0.900$), regardless of the plant cultivar and the oil sample source. The results presented could serve as a good basis for developing authentication methodologies of commercial pumpkin seed oils on the market, considering that such high-values food products are prone to adulteration practices [5].

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Linking cocoa polyphenol composition to chocolate quality with Average-Mass-Spectra fingerprints

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Approaches enabling prediction of chocolate quality from cocoa composition would avoid time- and money-consuming steps to chocolate makers. Average mass spectra of cocoa-polyphenol-extracts led to fingerprints used to select the molecules that discriminate chocolate sensory groups.

Sixteen worldwide cocoa samples were processed into chocolates which were characterized by sensory analysis, allowing sorting of the samples into four sensory groups.
The cocoa polyphenol extracts were analyzed by liquid chromatography−low-resolution mass spectrometry. Averaging each mass spectrum provided polyphenolic fingerprints, which were combined into a matrix and processed with chemometrics (PCA, PLS-DA) to select the most meaningful molecules for discrimination of the chocolate sensory groups.

A larger set of 44 cocoa samples was used to validate the previous results. 29 mass signals of known and unknown molecules, mainly flavan-3-ols, were finally targeted[1], including 2 newly described ethyl-bridged flavan-3-ols[2], enabling sensory-group discrimination. Average mass spectra fingerprints of cocoa-polyphenol-extracts proved to be quick and efficient to select the molecules that discriminate chocolate sensory groups.

A targeted MRM (Multiple Reaction Monitoring) mass spectrometry method was then developed and validated to routinely analyse large series of cocoa samples.

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Criteria for selection of antioxidant capacity assays for food antioxidants: Evaluation of CUPRAC, FRAP, Folin-Ciocalteau, ORAC, ABTS and DPPH assays

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The CUPRAC (CUPric Ion Reducing Antioxidant Capacity) assay, launched in 2004 by our research group, has served the scientific/technological community for the last 15 years in determining the total antioxidant capacity (TAC) or activity of food and biological antioxidants [1]. CUPRAC is a versatile method capable of determining both hydrophilic and lipophilic antioxidants in perfectly linear absorbance-concentration relationships, because the CUPRAC reagent, cupric-neocuproine chelate, has an affinity toward both the aqueous phase and organic solvents, and a single chromophore, cuprous-neocuproine chelate absorbing at 450 nm, forms at the end of the redox reaction with antioxidants. Besides, CUPRAC has favourable kinetics and optimal redox potential toward most food and biological antioxidants for which FRAP (ferric reducing antioxidant power) method is less responsive. CUPRAC well responds to small-molecule and protein thiols with which FRAP reacts much more slowly. CUPRAC has been transformed into a membrane-sensor format [2], and coupled to both online-HPLC and nanotechnological [3] methods. The exact redox potential of the Folin-Ciocalteau reagent, phosphotungstomolybdate(VI), is unknown, causing the undesired oxidation of citrate, several amino acids and sugars (*i.e*., not classified as true antioxidants) with this reagent. ORAC (oxygen radical absorbance capacity) assay makes use of a fluorescence decay curve of a peroxyl radical–attacked probe (fluorescein) in the absence and presence of antioxidants, in which a retarded fluorescence decay is observed with peroxyl scavengers. ORAC is advantageous in measuring both the extent (efficiency) and kinetics of antioxidant action with an area-under-curve (AUC) approach to the fluorescence decay curve. However, ORAC may suffer from possible polyphenol interactions with other food constituents giving rise to large deviations of found *versus* expected results, and certain amino acids may show quite high or low ORAC values. Steric accessibility of the radical reagent, when combined with kinetic solvent effects, may give rise to serious limitations in TAC measurements with ABTS and DPPH radicals [4]. Moreover, the ABTS radical generating oxidant, persulfate, can be easily activated by transition metal ions to exhibit its strong oxidant effects that may interfere with TAC measurement, a property not previously recognized.

It may be argued that a carefully standardized TAC method should utilize a biologically relevant or simulated oxidizing agent, be simple, reasonably rapid, reproducible and versatile, have a clear-cut end point, have readily available reagents and equipment, and not involve redox cycling of end products (derived either from the antioxidant or reagent) in order to give meaningful results. In this presentation, the widely used TAC assays, especially those based on electron transfer (ET) reactions, will be evaluated and compared together with their controversies and limitations, and a practical guide for the potential user will be provided in regard to method selection to be used in complex media serving a specific purpose.

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Trends in human diets: Seeds as a source of fibre

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Currently consumers search for healthier food options, prioritizing an adequate intake of nutrients and possible therapeutic effects. Seeds, which were previously consumed in the past, are now becoming a common trend among human diets. Besides fat and protein, they are an important source of dietary fibre.

Dietary fibre is a complex group of substances, providing several functional health benefits: laxative effect and improvement in blood glucose and cholesterol levels. However, different dietary fibre types exert different physiological properties, influencing gut microbiota composition [1]. It is highly influenced by the diet and any change will exert a dynamic impact on its abundance and diversity. Therefore, an unbalanced diet may affect the microbiome which has a significant and complex role in human metabolism, leading to a disequilibrium called dysbiosis and, consequently, to diseases, such as, irritable bowel syndrome and obesity or, in the worst cases, cancer [2].

Therefore, the aim of this work was to characterize seeds commonly available and currently consumed, namely, whole chia, flax, poppy and sesame seeds, through performing a nutritional analysis, following AOAC procedures. Moisture, ash, fat, protein, total dietary fibre (TDF) and insoluble fibre were determined [3]. In order to meet consumer's demands and preference for diets with low fat contents, the cake (by-product obtained after oil extraction by cold-pressing at 4 ^{o}C) was also evaluated.

Concerning the results, as expected, the cakes were enriched in protein and fibre as they lost most of the oil portion, apart from sesame which presented the lowest oil yield. Chia cake and whole seed presented the highest contents of TDF (more than 40% d.w.). On the contrary, sesame cake and seed presented the lowest values (less than 25% d.w.), what is due to the higher amounts of fat in these samples (>30 and >50% d.w., respectively).

To conclude, the cake evidenced great potential for incorporation in various food products, for instance, baked goods, energy bars or even yogurts, as it is a high source of protein and especially dietary fibre. The whole seeds, besides protein and fibre, also present high contents of fat, which can provide essential fatty acids and vitamin E contents, being suitable to be included in a healthy and varied food pattern.

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ORAL COMMUNICATIONS

Functional Foods

Prenylated flavonoids derived from *Flemingia philippinensis* display potent bacterial neuraminidase inhibition activities

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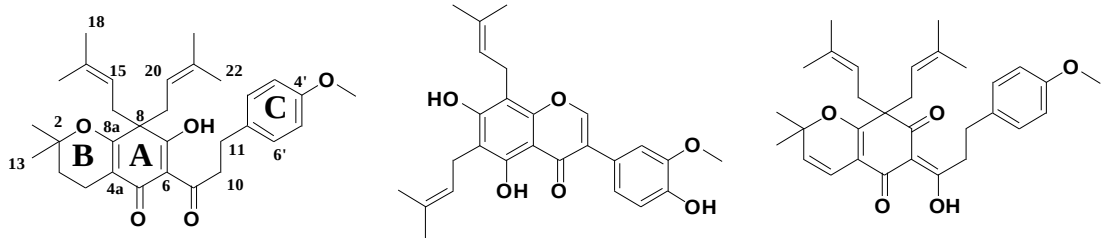
Prenylated flavonoids are special type of polyphenol compounds which exists in common plants such as hops, jackfruit and plants from leguminosae family. *Flemingia* is a popular edible species, which belongs to the legume family and have been cultivated in the tropical parts of China as an important food ingredient and medicinal plant. The organic extract of the roots of *Flemingia philippinensis* showed high bacterial neuraminidase (NA) inhibitory activity

NA is one of the key enzymes involved in pathogenesis of inflammation during infection and subsequent. Activity-guided separation of the methanol extract yielded different types of prenylated flavonoids. Isoflavone and chromenone derivatives are main types of compounds. Especially, three new compounds were purified with other 10 known prenylated flavonoids. All prenylated flavonoids isolated showed potent bacterial neuraminidase inhibition activites and their IC_{50} values were determined to range between 0.07 and 56.8 μM. In kinetic studies, isoflavones and 4a,8-diprenyl chromenone compounds showed non-competitive behaviours whereas 8,8-diprenyl chromenone compounds displayed competitive inhibitory mode. The prenyl groups greatly affected inhibitory behaviour towards genistein and quercetin.



Philippin D **Flemingsin** **Philippin E**

Fig.1. Chemical structures of isolated new isoflavone and chromenone derivatives from *F. philippinensis.*

Table 1. Inhibitory effects of new compounds on neuraminidase activities

Compound	IC_{50}	Kinetic mode (K_i, μM)
Philippin D	0.75 ± 0.07 μM	Competitive (0.46 ± 0.07)
Flemingsin	3.75 ± 0.4 μM	Noncompetitive (3.68 ± 0.5)
Philippin E	0.54 ± 0.05 μM	Competitive (0.31 ± 0.02)
Genistein	12.05 ± 1.3 μM	NT[a]
Quercetin	18.7 ±0.94μM	NT[a]

[a] NT is not tested.

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Influence of the harvest stage on the phenolic composition and bioactive properties of *Cynara cardunculus* L. var. *altilis* heads

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Cynara cardunculus L., commonly known as cardoon, is an erect perennial herbaceous plant. It belongs to the *Asteraceae* family and it is native to the Mediterranean area. This species comprises three taxa: the wild cardoon (var. *sylvestris*), the domesticated cardoon (var. *altilis*) and the globe or head artichoke (var. *scolymus*) [1]. Their edible capitula are widely used in several food recipes and as an herbal medicine, due to their known health-promoting effects and richness in bioactive compounds. The diverse industrial applications attributed to this crop (*e.g.* cheese manufacturing, biomass production, bioenergy and solid biofuels production, and pharmaceutics) make its cultivation highly important and with economic impact [1-3].

Thus, due to its increasing consumption and commercial interest, the present study purposes the analysis of phenolic compounds and bioactive properties of cardoon heads at different harvest stages. Cardoon (var. *altilis)* head samples were collected in Greece, at five different harvesting times, presenting therefore different maturation stages. The phenolic composition was determined in hydroethanolic extracts by HPLC-DAD-ESI/MS. The antimicrobial activity was tested against three Gram-positive and three Gram-negative bacteria, and six fungi species using the microdilution method. The cytotoxic effects were evaluated against four human tumor cell lines and in a porcine liver primary cell culture using the sulforhodamine B assay. The anti-inflammatory activity was evaluated through the inhibition of NO production using lipopolysaccharide-stimulated macrophages. Finally, the antioxidant activity was measured by TBARS and OxHLIA assays.

Nine phenolic compounds were tentatively identified, with *cis* 3,4-*O*-caffeoylquinic acid and apigenin-*O*-glucuronide being present in higher quantities. The content of phenolic compounds decreased with maturation process; the latest harvest caused a loss of 78% of the phenolic content. All the tested samples exhibited antibacterial and antifungal activity; but unlike the findings for phenolic content, the mature heads revealed the lowest MICs. Regarding the cytotoxic activity, the earliest harvest (immature heads) revealed activity against all the tumor cell lines tested, except for breast cancer cell lines for where the latest harvest had the highest potential. Moreover, the mature head extracts presented capacity to protect erythrocytes from the free radicals generated in the reaction system. Regarding the anti-inflammatory activities and TBARS inhibition, the immature heads revealed the highest activity. The heterogeneity of the biological results reveals that other compounds than phenolic ones may be correlated with these bioactivities.

This study proved the high biological potential of cardoon heads as also its possible use as a source of important bioactive compounds. Nevertheless, further studies are needed to understand which compounds are responsible for the observed bioactivities, as well as to find the stage of maturity that provides the best bioactive properties.

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Study of functional properties of wild and cultivated edible mushroom powder

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Mushrooms have attracted much attention in balanced diets not only because of their nutritional value but also because they contain functional compounds (proteins, polysaccharides, phenolic compounds, etc.) with potential beneficial effects [1]. Nutritional composition and functional properties of six wild, *Cantharellus cinereus, Calocybe gambosa, Cratherellus cornucopioides, Hygrophorus marzuolus, Lactarius deliciosus, Boletus edulis* and two cultivated, *Pleurotus ostreatus, Agaricus bisporus*, mushroom powders have been determined in order to be used in new fortified food formulations.

Fruiting bodies of these mushrooms collected or bought in Soria (Spain) were freeze-dried and finely milled. Nutritional analyses of mushroom powder were conducted following AOAC methods [2]. Functional properties were determined according to *Chau et al.* [3]: pH; apparent density (AD); oil absorption capacity (OAC); water absorption capacity (WAC); absorption capacity (AC); swelling capacity (SC); emulsifying ability (EA); emulsifying stability (ES); least gelation concentration (LGC) and foaming capacity (FC).

By studying the obtained results, for instance, the significant difference of swelling capacity of *C. cornucopioides,* the least gelation concentration of *B. edulis* and the foaming capacity of *A. bisporus*, new formulations of healthier foods are being developed.

Table 1. Functional properties of eight edible mushrooms. Mean (n=2). SD (Standard Deviation)

		pH	AD g/mL	OAC mL/g	WAC mL/g	AC mL/g	SC mL/g	EA %	ES %	LGC %	FC %
Cratherellus cornuco_ pioides	*Mean*	5.995	0.689	1.729	5.466	3.701	8.561	7.636	7.636	12.00	4.000
	SD	0.049	0.008	0.202	0.131	0.124	0.310	0.514	0.514	0.000	0.000
Calocybe gambosa	*Mean*	6.465	0.424	3.226	5.099	3.617	4.655	3.272	2.909	8.000	29.40
	SD	0.064	0.033	0.042	0.142	0.318	0.229	0.514	0.514	0.000	1.980
Pleurotus ostreatus	*Mean*	6.130	0.460	2.999	3.437	4.241	5.924	17.79	12.02	8.000	11.60
	SD	0.014	0.022	0.129	0.084	0.180	0.099	0.548	0.994	0.000	0.566
Cantharellus cibarius	*Mean*	5.835	0.352	3.892	3.692	3.875	5.335	5.273	5.273	8.000	16.00
	SD	0.007	0.016	0.164	0.151	0.032	0.333	0.257	0.257	0.000	2.828
Hygrophorus marzuolus	*Mean*	5.950	0.556	2.240	4.622	2.705	2.683	21.65	21.65	16.00	8.600
	SD	0.071	0.002	0.147	0.221	0.068	0.405	0.238	0.238	0.000	0.848
Lactarius deliciosus	*Mean*	6.115	0.378	2.854	4.878	3.663	5.551	24.32	17.11	12.00	23.00
	SD	0.078	0.011	0.082	0.452	0.214	0.262	0.964	1.056	0.000	1.414
Boletus edulis	*Mean*	5.625	0.499	2.572	5.991	3.486	0.861	21.15	21.15	4.000	9.600
	SD	0.007	0.008	0.285	0.279	0.039	0.056	0.000	0.000	0.000	0.566
Agaricus bisporus	*Mean*	6.640	0.210	4.285	4.293	4.214	1.884	21.15	17.88	4.000	10.00
	SD	0.014	0.023	0.435	0.005	0.351	0.146	0.000	0.816	0.000	0.000

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Development of a new functional dairy product enriched in phytosterols: the importance of food chemistry

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Among the chronic diseases related to the human diet, cardiovascular disease, which is associated to high levels of total cholesterol, is one of the most leading cause of death in the world [1]. Nowadays, the growing consumer awareness of their blood cholesterol level, has guided the food industry to respond by proponing new food products, which could satisfy consumer expectations. Therefore, during the recent years, the development of low-fat food products has considerably increased especially in the dairy sector. Another strategy has consisted to develop functional dairy products (F.D.P) containing phytosterols, which are steroid compounds occurring in plants and having a lowering effect on blood LDL-cholesterol [2]. This development of new functional food (F.F) is an interconnected process involving food industry companies, European commission regulations, marketing and scientists [3]. In this regard, researchers from the University of Camerino and a cheese producing company named SABELLI have come together to produce a new phytosterols-enriched ricotta cheese. To our knowledge, it is the first time that a ricotta cheese enriched in phytosterols is developed.

The process leading to this new F.D.P has been a complex pathway with many challenges. Thus, the present report aims to show step by step all the food chemistry studies performed to realize this product. To obtain this F.F, we started by [a)] studying the nutritional composition of ricotta in order to assess its possible health claims; then, we have to challenge [b)] the development and the validation of a new low-cost and fast analytical method for phytosterols quantification using high performance liquid chromatography-diode array detector (HPLC-DAD). However, due to their lack of chromophore group and their low ionization in mass spectrometry (MS), we have to innovate by developing a new derivatization method using dansyl chloride which allowed to detect plants sterols and stanols at 254 nm in DAD and highly increased their ionization in MS. The third step consisted to [c)] study the composition and the stability of phytosterols ingredients during ricotta production and storage. After validation, we finally [d)] studied different formulations for ricotta enrichment and assessed the effect of this enrichment on the organoleptic properties and the quality of the ricotta after its production and during its shelf-life.

These studies illustrate in a practical way, the crucial role of food chemistry in functional foods development and therefore, the impact of this research field in the food industry growth and the improvement of the human health.

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Administration of *Castanea sativa* flowers extract in Wistar rats

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Castanea sativa Mill. flowers (CF) are reported by ancestral claims as having health benefits like mucolytic, antispasmodic and anti-dysenteric properties and, in vitro studies showed anticancer properties against breast, colon, cervical and hepatocellular carcinomas [1].
The aim of this work was to study the effect of chestnut flowers (CF) extract on rat's physiological parameters. Fifteen male Wistar Unilever rats were randomly divided into two groups: untreated control, n=10, and CF group, n=5. Animals from CF group were exposed to the chestnut flowers extract in drinking water (20 mg/animal/day) for 49 weeks. Body weight, food and drink consumption were measured weekly. At necropsy, all organs were collected, weighed and liver and kidney were processed for histological analysis. Animals from CF group showed a mean final body weight and a food consumption higher than untreated animals ($p>0.05$). Animals from CF group showed lower consumption of water ($p=0.000$). Relative mean liver weight from animals treated with CF was higher than untreated animals ($p=0.026$) and presented more liver degeneration. However, liver inflammatory infiltrate was reduced ($p=0.026$) in animals exposed to CF extract. There were no significant differences in relative mean kidney weight among groups. Untreated animals developed 50% of discrete proteinuria and CF treated animals developed 20%. Serum levels of albumin, total protein, glucose and alanine aminotransferase did not show significant differences between experimental groups.
These results suggest that chestnut flowers extract was well tolerated by the animals, did not cause hepatic and kidney toxicity and had no effect on biochemical profile. Further studies are necessary to evaluate in vivo, the effect of different CF doses and evaluate the respective potential use.

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Comparative effects of dietary hempseeds and hempseed oil on liver functions and lipid metabolism in genetically obese rats

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Seeds of industrial hemp (*Cannabis sativa L*.) contains approximately 30% of oil that is rich in polyunsaturated fatty acids (PUFA), including essential fatty acids and others, which can affect lipid metabolism and improve cardiovascular health. Hempseeds are also a good source of dietary fibre and contain other non-nutritive compounds, such as lignanamides and phytic acid, all of which are biologically active as well [1]. Available studies have been focused either on nutritional properties of hempseeds or cardioprotective properties of hempseed oil and other components isolated from them[2,3,4]. However, the extent to which PUFA can contribute to the health effects of hempseed consumption has not been studied thus far. Therefore, the aim was to compare the effect of dietary supplementation with hempseeds and hempseed oil on liver functions and lipid metabolism in genetically obese Zucker rats. We hypothesized that an adequate hempseed or hempseed oil supplementation can attenuate genetic-related disorders and the former are more efficient in doing so.

The feeding experiment was conducted on 30 lean (Fa/?) and obese (fa/fa) Zucker rats allocated to four groups. The lean control (LC) and obese control (OC) groups were fed a standard, semi-purified diet, which contained, among others, casein, cellulose and rapeseed and palm oil (4% diet each) as the source of protein, fibre and fat, respectively. The other two obese groups were fed a modification of the standard diet, in which hempseed oil was added at the expense of palm oil (4% diet; group OHO) or grinded hempseeds were added at the expense of palm oil, cellulose and casein (12.05% diet; group OHS). All diets had the same proportion of protein, fibre and fat, whereas diets fed to the OHO and OHS groups had similar fatty acid profile, including more than two times increased proportion of PUFA that originated either from hempseeds or hempseed oil.

After 4 weeks of experimental feeding, the OC group was characterized by the increased body weight, body fat percentage and liver mass, including increased liver fat, triglyceride and cholesterol contents. Moreover, the hepatic expression of peroxisome proliferator-activated receptor (PPAR) α and γ was decreased, whereas the plasma aminotransferase activities and the plasma cholesterol and triglyceride concentrations were increased in the OC group compared to the LC group. Neither of dietary factors was able to affect the body weight and body composition of rats. Dietary supplementation with hempseeds decreased liver mass, whereas both dietary factors reduced the liver cholesterol content and increased hepatic PPARγ expression; however, the increase in the expression was especially visible in the OHS group. Moreover, dietary hempseeds decreased the plasma aminotransferase activities and triglyceride and cholesterol concentration (group OHS), whereas hempseed oil was only able to decrease the plasma triglyceride level (group OHO).

In conclusion, this study demonstrates that both hempseeds and hempseed oil can attenuate genetic-related disorders in liver functions and lipid metabolism; however, they are not able to affect the development of obesity itself. Dietary supplementation with hempseeds is more efficient in limiting lipid disorders and improving liver functions in Zucker rats than with the oil extracted from them, which suggests that PUFA are only partly responsible for these beneficial effects.

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Acorn Flour as bioactive compounds source in gluten free bread

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Acorn is the fruit of *Quercus* gender tree, and the most common in human feed are from *Quercus ilex*, *Quercus rotundifolia* (holm oak) and *Quercus suber* (cork oak). Both *Quercus* have origin in South Europe and are abundant in Portugal, Spain, France and Italy. Its nutritional characteristics are really interesting: rich in unsaturated fatty acids and fibre, vitamin E, chlorophylls, carotenoids total phenolics and antioxidant activity, besides is naturally gluten free [4].
Acorn flour is known as ingredient to bake bread since Roman times. Along the history it was used specially in scarcity years. With the arriving of maize and potatoes from South America, its consumption was almost lost. Nowadays it is a high valuable resource, used to feed Iberian pig breed, but about 50% of the production available isn't used and is being lost [1;3].
In the last years, gluten free bakery has increased its consume, not only for celiac patients, but also as market trend. Generally, gluten free bread is nutritionally poorer, what brings many deficiencies to celiac patient's diet and consequently constrains in their health [2;5].
Recognizing acorn flour as bioactive compound and following the tradition of its using to bake bread, the use of this flour as a natural gluten free ingredient to bake gluten free bread was studied. Breads were prepared with buckwheat, rice, acorn flours and potato starch. Two levels of acorn flour (23% and 46%) were tested and both sensorial and nutritional results, with special attention to bioactive compounds in the final product were compared [4].
Bread physical and sensorial characteristics were analysed: texture (TA.XT.plus, Stable Micro Systems), colour (Konica Minolta), volume (AACC), water activity (Rotronic-HygroPalm) and staling (texture and colour during aging period). About bioactive compounds, total phenols, *orto*-diphenols, and flavonoids were evaluated as well as the phenolic profile that was performed by HPLC. Also antioxidant activity was determined by ABTS, DPPH and FRAP methodologies.
Acorn flour showed good technological properties as food ingredient in gluten free bakery, contributing to the aim of improving bread nutritional and sensorial characteristics.

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The flours were offered by the companies: Terrius, Próvida, Fábricas Lusitania and A Colmeia do Minho

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The effect of diets supplemented with hemp and poppy seed oils on the development of obesity-related disorders in Zucker rats

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Currently hemp and poppy seed oils are produced and marketed as functional products with health-promoting potential. The increased interest in these oils is mostly due to their high content of essential fatty acids. It is known that these fatty acids play an important role in the regulation of lipid metabolism and they can mitigate obesity-related disorders. Most of the studies on hemp and poppy seed oils have been focused on their chemical analysis and only some of them on their cardioprotective effects. There is lack of experiments on potential benefits of these oils in the regulation of obesity-related disorders. Therefore, the aim of this experiment was to compare the effects of dietary supplementation with hemp and poppy seed oils on the activity of intestinal microbiota, liver disorders and fat accumulation in Zucker rats with genetic predispositions to gain obesity.

Unrefined cold-pressed hemp and poppy seed oils were of commercial origin. Fatty acid profile of oils were analysed by conversion of fatty acids to methyl esters. Afterwards, the analysis was performed using gas chromatography. The nutritional experiment was performed on male Zucker fatty rats allocated to 3 groups of 8 animals each with similar initial body weight. Animals were fed for 4 weeks with modified rodent diets (AIN-93). Each diet contained 7% of different oils: palm oil (group C), hemp seed oil (group H) and poppy seed oils (group P). After experimental feeding, the animals were sacrificed and biological material was taken for the analysis of short chain fatty acids (SCFA) in the caecum, liver fat and oxidative stress, plasma lipid profile and body fat accumulation.

Hemp and poppy seed oil were especially rich in polyunsaturated fatty acids (PUFA, 68% and 76%, respectively). The hemp seed oil contained mostly linoleic acid (53%), α-linolenic acid (17%) and γ-linolenic acid (4%), whereas poppy seed oil contained linoleic acid (67%) and α-linolenic acid (1%). After 4 weeks of feeding there were no significant differences in diet intake and body weight among experimental groups. Compared to the C group, the body fat content and hepatic cholesterol content were significantly reduced in H and P groups. Nevertheless, the supplementation with examined oils increased oxidative stress in the liver, including increased concentration of malondialdehyde (MDA) and decreased ratio of reduced to oxidized glutathione (GSH/GSSG). The oxidative stress induced by these oils might be associated with observed hepatic fat overload in Zucker rats and the increased level of polyunsaturated fatty acids, which are potential substrates for MDA production. Compared to the C group ,the plasma lipid profile was characterized by unfavorable reduction of HDL cholesterol in the H and P group, as well as the increased concentration of LDL cholesterol in the P group. Among the examined groups, only group H showed a significant reduction of plasma triglycerides (TG). Moreover, the abovementioned changes in plasma lipid profile corresponded to some extent with changes in the microbial production of SCFA in the caecum. Only group H was characterized by reduced production of acetic acid and finally by decreased concentration of total SCFA, which are known to regulate liver fat metabolism. The acetic acid promote lipogenesis in the liver, therefore its lower production can partially explain the observed lower plasma TG concentration in group H.

To conclude, the study on obese Zucker rats has been shown that 1) hemp and poppy seed oil are a valuable source of PUFA and can beneficially reduce body fat content and hepatic cholesterol level; 2) hemp seed oil favorably reduced plasma TG and regulated bacterial SCFA production in the caecum; 3) both oils caused oxidative stress in the liver.

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Enhanced extraction of ergosterol from *Pleurotus ostreatus* using response surface methodology (RSM)

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Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm., is one of the most widely consumed mushrooms in the world with interesting health-promoting benefits, mainly due to its richness in several bioactive compounds [1]. Mushrooms produce ergosterol as one of their main sterols, which has been considered a contributor to their anti-inflammatory, antioxidant, and antitumor properties [2]. Obtaining an ergosterol enriched extract depends on different variables, such as the extraction method, solvent type, temperature, extraction time, and the solid-liquid ratio [3]. Therefore, it is essential to define the main variables and relevant response criteria to maximize the extraction yield and purity, combining the economic competitivity.
In the present work, response surface methodology (RSM) was applied to optimize a heat assisted extraction system (HAE), combining time (*t*) and temperature (*T*) effects, and using a circumscribed central composite design (*CCCD*) for the recovery of ergosterol from the fruiting bodies of *P. ostreatus produced with lignocellulose substract*. The obtained responses were the quantification of ergosterol by HPLC-UV (Y_1: mg of ergosterol per g of extract residue and Y_2: mg of ergosterol per 100 g of dry weight mushroom), and the extraction yield (Y_3: %). The *CCCD* consist of 16 response combinations and 4 centre points. Response surface models were fitted by using the following second order polynomial equation:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j + \sum_{i=1}^{n} b_{ii} X_i^2$$

The results obtained showed a significant interaction between the variables. For all the three responses (Y_1, Y_2, and Y_3), the model successfully explained more than 80% variation in the experimental data (i.e. R^2>8). The individual optimum conditions and responses were as follows; Y_1 (10 min, 30°C, 57.6 mg/g), Y_2 (150 min, 61°C, 246.3 mg/100 g dw), and Y_3 (10 min, 80.9°C, 9.3%). The global optimum conditions predicted by the model were: 150 min and 54.3 °C, capable of yielding 7.3 %, 33.3 mg/g and 244.3 mg/100 g dw. The values predicted by the model are in close agreement with the experimental observations with very low residual distribution, proving the validity of the applied model. The results also showed the usefulness of the predictions for future scale up based on the desired responses. The obtained ergosterol enriched extract can be considered as a bioactive ingredient for pharmaceutical, cosmeceutical and nutraceutical purposes.

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Biological activities of stilbenoids *in vitro*

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Background and objectives: *Vitis vinifera* L., which belongs to the family Vitaceae, is rich in stilbenoids, comprising monomers, dimers as well as oligomers. Stilbenoids, especially resveratrol, potentially convey health benefits like anti-oxidant, anti-inflammatory, anti-cancerogenic and anti-atherogenic [1]. To date, numerous *in vitro* and *in vivo* studies have been performed with pure resveratrol. However, our diet consists of different secondary plant metabolites, and synergistic effects of the various stilbenoids may have an impact on their bioactivity. Therefore, in the present study, we investigated the stilbenoid composition and *in vitro* biological activity of a standardized stilbenoid-rich *Vitis vinifera* root extract [2].

Methods: The qualitative and quantitative stilbenoid composition was analyzed by HPLC-ESI-MS/MS and HPLC-PDA. The ability to prevent of H_2O_2-induced DNA damage was confirmed by the Comet assay. Nrf2 transactivation and induction of the Nrf2 target genes heme oxygenase (HO-1) and γ-glutamylcysteine-synthetase (γ-GCS) was determined by reporter gene assay, real-time PCR and Western blotting, respectively, in human liver cells. Biomarkers of inflammation, including interleukin-1β (IL-1β) and inducible nitric oxide synthase (iNOS) were measured in LPS-stimulated murine macrophages by real-time PCR.

Results: Seven stilbenoids, including two monomeric (resveratrol and piceatannol), two dimeric (trans-ε-viniferin and ampelopsin A), one trimeric (miyabenol C) and two tetrameric (r-2-viniferin and r-viniferin) compounds were identified in the *Vitis vinifera* root extract. The root extract protected against H_2O_2-induced DNA damage and, in the hepatocytes, it induced the transcription factor Nrf2 as well as its target genes HO-1 and γ-GCS. Furthermore, in macrophages, the roots extract down-regulated pro-inflammatory gene expression, such as of IL-1β and iNOS.

Conclusions: The main compounds in the *Vitis vinifera* root extract were the dimer trans-ε-viniferin (125 g/kg), the tetramer r2-viniferin (87 g/kg), and the monomer resveratrol (46 g/kg). The root extract possessed anti-oxidant and anti-inflammatory properties *in vitro*. In future studies, the bioactivity and bioavailability should be substantiated in *in vivo* studies. The *Vitis vinifera* root extract could also be of interest for the food industry, e.g. as one means to avoid lipid peroxidation in fatty products.

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Simultaneous quantification of 30 different bioactive compounds including polyphenols in spent coffee ground and coffee silverskin by HPLC-MS/MS triple quadrupole

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Coffee is one of the most consumed beverages worldwide and according to the latest statistics the global consumption is more than 150 million of 60 kg coffee bags per year. As a consequence, large amount of coffee residues need to be disposed of [1]. Although many studies related to coffee and health have been carried out in green, ground and espresso coffee, two components of the coffee processing chain have been less investigated, i.e. coffee silverskin (CS) and spent coffee ground (SCG), the two main by-products presenting a huge potential to be valorized [2]. It is well known that coffee is a rich source of polyphenols, phytochemicals associated with many biological activities, and they are partially extracted from the beans during brewing; so, the resulting spent coffee is still a rich source of polyphenolic compounds. Similarly, silverskin is also considered to be a potential source of polyphenolic compounds because this tegument is in direct contact with the beans; therefore, it keeps part of the polyphenolic compounds constituents present in coffee beans [3].

On these bases, general aim of this project was to deepen the knowledge on CS and SCG, in the future perspective of their re-use as nutraceuticals by a) developing an efficient and low-cost method to extract different bioactive compounds including polyphenols from these by-products b) quantifying these bioactive compounds (chlorogenic acids, caffeine and other important coffee polyphenols such as flavonoids and phenolic acids) from different CS and SCG extracts by developing new HPLC-MS/MS analytical method.

SCG and CS samples were extracted by using different solvents and extraction procedures (i.e. magnetic stirrer, ultrasound extraction). From an analytical point of view, UHPLC–MS/MS studies were performed using an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology equipped with an ESI source operating in negative ionization mode. The separation was achieved using a Kinetex C18 analytical column (50 mm × 2.10 mm i.d., 2.6 µm) using a binary gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The method was sensitive (LOQs for all compounds were in the range 1-100 µg kg^{-1}), linear (R^2 for all analytes was higher than 0.9907), accurate and robust. The best extraction procedure in term of amount of bioactive compounds extracted was ultrasound extraction with a mixture of ethanol:water 70:30 *v/v*; in this case, the highest amount of total bioactive compounds concentration was found.

In the future, the most promising extracts in terms of bioactive compounds will be tested to evaluate theirs prebiotic and antimicrobial activity, in the perspective of their re-use as nutraceuticals.

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In vitro bioaccessibility and bioavailability of minerals and pigments from macroalgae-fortified frankfurters

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Meat products are known as a rich source of valuable nutrients, although when consumed in inappropriate quantities, may enhance the risk of some diseases [1]. In turn, the increasing consciousness of consumers on their eating habits has been boosting naturally-derived products demand. Hence, the development of meat-based functional products through the incorporation of healthier ingredients such as macroalgae may offer an interesting opportunity to address consumers' needs and to update recommendations regarding nutritional and dietary goals.

In this context, the nutritional composition of two fortified frankfurters (F1 and F2) formulated with distinct percentages of green, red and brown macroalgae was assessed. The major effects resulting from macroalgae fortification were observed in ash (3.0-3.6 %FW) and fiber (1.0-1.2 %FW) levels, which were higher than the control (2.0 and 0.5 %FW, respectively). Further, the new food products were characterized by a lower Na/K ratio and superior levels of I, Fe, K, and Mg, while minor impacts were observed for the remaining nutrients. Afterwards, fortified frankfurters F1 and F2 were subjected to different static *in vitro* digestion methods [2,3] in order to evaluate the bioaccessibility and bioavailability of the major incremented compounds, namely minerals and pigments. As accessed for F1 frankfurters, K, Mg, P, I and Zn were available for absorption after gastrointestinal digestion, which % bioaccessibility ranged between 18-100%. The use of dialysis membranes to mimic intestinal absorption revealed that K, Mg, P and I were potentially capable to be absorbed, showing % bioavailability from 10-50%. In addition, the effect of cooking (scalding) on mineral content was studied. As expected, mineral levels decreased slightly with cooking, which resulted in a decrease of 11-16% and 25% of bioaccessibility and bioavailability. Although, bioaccessible and bioavailable fractions of iodine suggested to be positively affected with cooking.

Pigments analysis in frankfurter F2 revealed the presence of chlorophyll pigments of the *a*, *b* and *c* series (126 mg/Kg FW), being pheophytin *a* and pheophorbide *a* the major ones. Carotenoids, mostly lutein and fucoxanthin, were also quantified (6 mg/Kg FW). *In vitro* digestion modified the chlorophyllic profile, being chlorophylls *a* and *c* completely transformed in Mg-free derivatives, while some chlorophyll *b* remained. Estimated bioaccessibilties of total chlorophylls and total carotenoids, defined as the percentage of the initial content in the frankfurter, were around 3.4 and 8%, respectively. In conclusion, the use of macroalgae as ingredient in frankfurters may be a potential form to improve their health benefits beyond the basic nutritional value, thus contributing to health and wellness.

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Influence of heat treatment on biological compounds profile and antioxidant activity of herbs and spices and cookies with their contribution

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Herbs and spices are a natural source of biologically active compounds and therefore, they possess strong antioxidant activity [1,2]. The content of these compounds, thus the antioxidant potential, depend on the manner of technological treatment and herbs and spice matrix content [3].

The aim of the study was to determine the effect of temperature on the profile of biologically active compounds and antioxidant activity in herbs and spices, and in cookies with herbs and spices additives.

The object of the study consisted of 10 selected herbs and spices (bay leaf, cinnamon, clove, tarragon, mayoram, mint, oregano, rosemary, sage and thyme), these herbs and spices after heat-treatment (85°C, 20 minutes), as well as oat-buckwheat dough and cookies with 2% addition of herbs/spices (baked at 180°C for 25 minutes). The total phenols content (TPC) was determined using the Folin-Ciocalteu reagent, while antioxidant properties were measured using photochemiluminescence (PCL) and FRAP methods. Furthermore, the profile of biologically active compounds was analysed by liquid chromatography combined with mass spectrometry (LC-MS/ MS).

The highest TPC value was observed in samples of cloves, mayoram and mint, whereas the highest antioxidant activity was noted in cloves, rosemary, sage and mint. Furthermore, an increase in TPC and antioxidant activity of herbs and spices after heat treatment was observed. On the other hand, in cookies with the addition of herbs and spices, almost 2-fold decrease of TPC, PCL and FRAP values were observed, in comparison to dough. In addition, 16 biologically active compounds, from the group of flavonoids and phenolic acids, in culinary herbs and spices, and cookies with herbs and spices addition, have been identified. Their contents vary depending on the type of herbs and spices used. However, in cookies a lower content of flavonoids and phenolic acids was found in comparison to their content in herbs and spices.

This study showed that heat treatment significantly influence on profile of biologically active compounds and antioxidant activity of herbs and spices, in raw material but also in different step of product preparation.

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Determination of the Interactions between Bound and Free Antioxidants Naturally Occurring in Foods

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Antioxidants constitute an important part of human nutrition due to correlation of their intake with lower incidence of cardiovascular diseases (CVD), cancer, ageing and related degenerative processes [1]. Besides being free from physical or chemical interactions, a significant amount of dietary antioxidants are also bound to different macromolecules like dietary fibers (DFs), proteins or lipids [2]. Since bound and free antioxidants constitutes a significant portion of human nutrition together, it is of great importance to examine the results of their consumption together. At this point, the interactions between free and bound antioxidants is essential to be investigated, in order to potentially take further advantage of their health effects after their consumption. If free and bound antioxidants are ingested together with foods, they can combine in any point of the digestive system, they may react, regenerate each other and create a synergistic effect. Antagonistic or additive interactions may occur as well. A synergistic interaction may result in a greater total antioxidant activity when compared to the simple sum of the antioxidant activities of bound and free antioxidants separately. Such kind of interaction may contribute to the healthy antioxidant environment in the gastrointestinal tract. On the contrary, an antagonistic interaction can create a total antioxidant effect that is less than the sum of the individual antioxidant activities, which may damage the existing antioxidant environment. On the other hand, an additive interaction may not affect the total antioxidant activity of bound and free antioxidants as they act separately [3].

The known regeneration reaction and synergistic interaction between free soluble and DF-bound antioxidants constitute the starting idea of this study [4,5]. Since a wide variety of free and bound antioxidants are available in our daily diet and various interactions may occur between them, a necessity for a broad study came out. In this context, the interactions between free and macromolecule bound antioxidants, obtained from different sources were investigated in this study. DF, protein and lipid-bound antioxidants, bread crust and coffee melanoidins were used as the bound antioxidants sources, while trolox, the reference antioxidant, catechins, hydroxycinnamic and hydroxybenzoic acids were used as the free antioxidant sources. Experimental studies were performed both in aqueous and liposome media to evaluate the effect of environmental conditions on the interactions between free and bound antioxidants. Antioxidant sources used in this study were carefully selected considering their consumption rates in daily diet. Hydroxycinnamic and hydroxybenzoic acids, containing different amounts of –OH and/or $–OCH_3$ groups localized at different positions of the aromatic ring were used in order to explain the interactions with a structural approach. Chemometric tools were used both at the experimental design and multivariate data analysis steps. Both synergistic and antagonistic interactions were observed depending on the free and bound antioxidant combinations. Characteristics of the lipid oxidation reactions in liposome medium and radical scavenging reactions in aqueous medium were centered with the Principal Component Analysis and the oxidation curves were successfully modelled. In conclusion, this study is an inception for further *in vivo* studies investigating the interactions between free and macromolecule-bound antioxidants after consumption in human body.

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Composition in anthocyanins and bioactive properties of jabuticaba bioresidues

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Myrciaria cauliflora (Mart.) O. Berg, known as jabuticaba, is a native species to Brazil, more specifically to Atlantic Rainforest biome. Its fruits are small berries with a diameter of 2.0 – 3.5 cm that contain between one and four seeds, with a sweet gelatinous pulp and thick dark purple epicarp when mature, which is not commonly consumed. Due to the properties of its pulp, the consumption and production of jellies and liqueurs from jabuticaba has increased. However, the epicarp that corresponds to about 50% of the fruit is not used, being converted into a bioresidue [1]. The intense colour of this part of the fruit is due to the pigments, namely anthocyanins, present in its composition. In addition to attractive staining, these molecules have been associated with bioactive properties, such as antioxidant activity, which makes their recovery interesting for application in various industrial segments, for instance, food and pharmaceutical [2,3]. With the objective of valorising this bioresidue, the present work aimed the determination of the main anthocyanins present in jabuticaba epicarp (by HPLC-DAD-ESI/MS) and the evaluation of the bioactivity of its ethanolic extract (through *in vitro* assessment of cytotoxic, antimicrobial, and antioxidant activities). Thus, cytotoxicity was evaluated in four tumour cell lines (NCI-H460 - lung carcinoma, MCF-7 - breast carcinoma, HepG2 - hepatocellular carcinoma, and HeLa - cervical carcinoma) and in a primary culture of non-tumour liver cells (PLP2), by the Sulforodamine B (SRB) assay. The antimicrobial activity was evaluated on five Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morgani, Proteus mirabilis*, and *Pseudomonas aeruginosa*) and three Gram-positive bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis*). Lastly, the antioxidant activity was tested through the oxidative haemolysis inhibition assay (OxHLIA).

The bioresidue of jabuticaba fruits presented two anthocyanins, identified as cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside, being the first one the most abundant. Regarding bioactivity, the ethanolic extract revealed antiproliferative activity in all tumour cell lines evaluated (GI_{50} < 300 µg/mL), except for NCI-H460, and did not show toxicity for PLP2 at the maximal tested concentration (400 µg/mL). It also exhibited bacteriostatic properties in all the analysed bacterial strains (ranging from 20 to 10 mg/mL). Regarding haemolysis inhibition, the extract was able to protect 50% of the erythrocyte population for 120 minutes in a lower concentration than the positive control (Trolox).

The results obtained in this study allow to conclude that jabuticaba epicarp is a rich source of anthocyanins and also exhibits strong bioactivity, which makes it suitable for use as colorant.

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Multistep fractionation of blackberry (*Rubus fruticosus* L.) pomace into high value functional ingredients

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Berries are among the richest sources of dietary antioxidants and other bioactive health beneficial compounds [1]. However, due to a very short shelf life significant part of berry harvests should be processed into various preserved products, generating huge amounts of by-products annually. Large amounts of berry pomace (by-product of juice pressing industry) currently are discarded as a waste, although retains a wide variety of bioactive constituents with potential food, pharmaceutical and nutraceutical applications [2]. The aim of this study – to develop a multistep biorefining scheme for recovery of various valuable substances from blackberry pomace by comparing the efficiencies of green innovative technologies, such as supercritical carbon dioxide (SFE-CO_2), pressurized liquid (PLE) and enzyme-assisted (EAE) extractions and conventional extraction methods.

Towards this end, SFE-CO_2 and PLE were optimized for effective non-polar and polar fraction isolation by response surface methodology (RSM) based on central composite design (CCD). Under optimal conditions (54.8 MPa, 64 °C, 171 min), SFE-CO_2 yielded 9.9% (w/w) of lipophilic fraction, rich in monounsaturated (oleic 14.5%) and polyunsaturated fatty acids (linoleic 64.1%, α-linolenic 12.9%). In a next step, PLE conditions were optimized to treat solid residues after SFE-CO_2. Optimized PLE gave 26.3 and 5.1 g of ethanol (10.3 MPa, 90 °C, 45 min) and water (10.3 MPa, 130 °C, 45 min) soluble pomace constituents, respectively. Thus, in total 41.3 g of various constituents (76% of the polar nature) were obtained from 100 g of blackberry pomace after high pressure fractionation, combining SFE-CO_2 with PLE with green food-grade solvents. These extracts contributed to 39.9 mg of gallic acid equivalents, 218.0 ($ABTS^{\bullet+}$) and 113.3 (ORAC) mg of trolox equivalents per 1 g of blackberry pomace. The major portion of the antioxidants were recovered by PLE-EtOH extract (73-80% in different assays), containing a number of phenolic acids, flavonoids and anthocyanins. Cyanidin-3-glucoside was the major anthocyanin detected both in pomace and its PLE extracts (4.8- 551 mg/100 g of pomace). The combined SFE-CO_2 and PLE reduced antioxidant capacity of starting plant material by up to 93%, showing that proposed biorefining scheme is efficient to recover the major part of the antioxidants from blackberry by-products. In terms of extraction time, solvent consumption, total yields, *in vitro* antioxidant capacity and phytochemical composition, maceration combined to EAE-Viscozyme treatment was less efficient to obtain target pomace constituents as compared to multistep high pressure fractionation.

In conclusion, the results of this work may be considered as another case study demonstrating that multistep high-pressure fractionation may be successfully applied for converting agro-food processing by-products and/or waste into the high value functional ingredients with multipurpose applications.

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Valorisation of a Portuguese endemic species as a potential functional food: *Thymus carnosus* Boiss

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Portugal's excellent geographic location favours the development of various medicinal plants, remaining the phytochemical composition of most of these species still poorly described. Until recently, this was the case of *Thymus carnosus* Boiss., a Portuguese endemic species that, due to its reduced geographical dispersion, is listed as endangered. The analysis of this species extracts, by HPLC-DAD and HPLC-ESI-MS^n, revealed a high content in phenolic acids (an isomer of salvianolic acid A, salvianolic acid K and rosmarinic acid) as well as terpenoids (ursolic and oleanolic acids), a composition that differs from other *Thymus* species. Additionally, *T. carnosus* extracts showed anti-proliferative, antioxidant and anti-inflammatory activity [1,2].

Aiming to revert the endangered status, a more comprehensive correlation of *T. carnosus* phytochemical composition and bioactivities is required, in order to justify the protection of this species and promote its cultivation for future inclusion in the diet as a functional food/ingredient.

T. carnosus aqueous and hydroethanolic (80% ethanol/water) extracts were applied to Caco-2 (Human colorectal adenocarcinoma) and HepG2 (Human hepatocellular carcinoma) cell lines. Both extracts induced time- and dose-dependent reduction in cell viability (Alamar Blue assay), with aqueous extracts being less cytotoxic and HepG2 cells being more resistant to the extracts effect. Reduction in cell viability is partially promoted by apoptosis, being the aqueous extract more effective than hydroethanolic extract (IC_{50} concentrations tested; Flow cytometry / Annexin-FITC and propidium iodide double staining). In Caco-2 cells, aqueous extracts induced morphological changes. And, both extracts induced cell cycle arrest, being the effect of aqueous extract in S and G2/M phases while hydroethanolic extract was in G0/G1 phase (Flow cytometry / Propidium iodide staining).

Therefore, anti-proliferative activity was observed for both extracts against two tumoral cell lines. The mechanism of cell death involves cell cycle arrest and programmed cell death. Morphological changes were observed, depending on the extract used. The differences between the observed biological effects can be correlated with the high concentrations of the pentacyclic terpenoids, ursolic and oleanolic acid, only present in hydroethanolic extracts. Thus, *T. carnosus* presents potential as a medicinal plant, justifying a more detailed study and its cultivation, before recommending its inclusion in Human diet.

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Structural analysis of *Nannochloropsis oculata* polysaccharides and its potential as functional food

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Marine microalgae are able to enhance the nutritional content of food preparations and promote the health of humans and animals. This is due to their capacity to produce biologically active compounds, such as proteins, lipids, polysaccharides and carotenoids. Nowadays, microalgae can be incorporated into pasta, snacks, candy bars, gums, and beverages [1]. *Nannochloropsis oculata* is a marine microalga not yet approved for human nutrition, but which produces the ω-3 eicosapentaenoic acid (EPA) that has recently been considered a dietary supplement ingredient [2]. This finding is regarded as a major boost to assess the use of *N. oculata* in human nutrition. Although the lipid composition of *N. oculata* is much studied, the polysaccharides composition remains unknown.

The present work aimed to determine *N. oculata* polysaccharides composition and structure, whose eventual relevance could justify further the consideration of this microalga for human consumption. To achieve this purpose, the polysaccharides were extracted with hot water and fractionated by ethanol precipitation and preparative size-exclusion chromatography. A combination of sugar and methylation analysis with data of carbohydrate microarrays disclosed the complex structural features of the different polysaccharides. *N. oculata* water-soluble polysaccharides are constituted by mixed-linked (β1→3, β1→4)-glucans and (α1→3)-, (α1→4)-mannans, which are described as dietary fibre with prebiotic activity [3]. Furthermore, it was also found a complex structure of anionic sulphated heteropolysaccharides composed of GlcA, Rha, Xyl, Gal, Fuc, and a sulphated rhamnan. The different soluble PS from *N. oculata* revealed also to stimulate murine B-lymphocytes *in vitro*, being the fraction enriched in sulphated heterorhamnans the one that promoted higher activation.

This work highlights that *N. oculata* water soluble polysaccharides show potential to be further explored for the development of functional foods.

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Response surface optimization of Phycobiliprotein pigments extraction from Gracilaria *gracilis* and application in pancakes

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Phycobiliproteins (PBPs) are non-toxic water-soluble pigment proteins that are mostly found in red algae, blue-green algae and *Cryptomonas*. Due to their strong absorbance and fluorescence properties and their antioxidant and free-radicals scavenging activities, PBPs have been widely employed in food, cosmetics, pharmaceutical and biomedical industries [1].

Red algae of the Gracilaria genus, are a rich source of PBPs, namely phycoerytrin (PE), and thus, they are valuable resources for industrial and biotechnological applications [2]. Gracilaria gracilis, a red algae found in the Portuguese coast, has been used in this study as a natural source for the extraction of PBP pigments for application as food colorant. The extractions were performed by treating freeze-dried algae with a phosphate buffer. Several extraction techniques, including maceration, ultrasounds, sonication, and freeze-thawing, have been tested and optimized. The extraction conditions, including phosphate buffer concentration (C), biomass:buffer solution racio (R), biomass:buffer solution contact time (t1), and extraction time (t2), were optimized using Response Surface Methodology (RSM). The highest yield of PE (measured spectrophotometrically) was obtained with maceration: 3.7 mg PE / g algae under the optimal conditions (C = 0.1 M, R = 1/50, t1 = t2 = 10 min). Scanning electron microscopy (SEM) was used to analyze changes in the cells morphology after extraction.

PBPs in the crude extract were purified through ammonium sulfate precipitation and dialysis and further applied as pigment in pankaces. A stability study was performed both on extracts and food product. Gracilaria *gracilis* is thus a good source of natural pigments for application in food products.

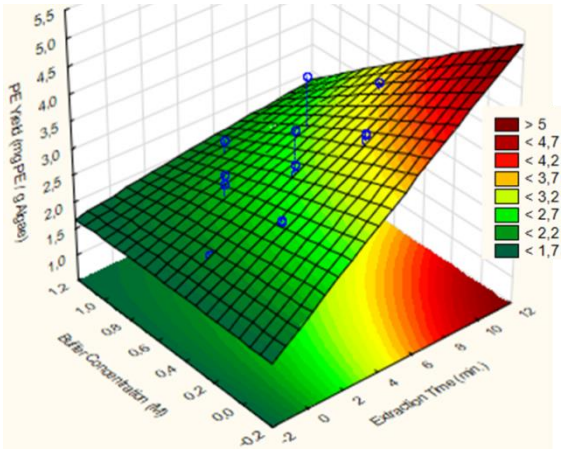

Fig.1. Response surface plot showing the influence of buffer concentration (M) and extraction time (min.) in the PE yield (mg PE / g algae) in maceration.

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Hemp Seed: an unthinkable source of bioactive compounds

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The link between health and food was established long ago, but only in recent years nutrition and nourishment have been re-proposed as an opportunity to prevent the most challenging health problems and manage the common ones. The attention of consumers is increasingly focused on a healthy lifestyle, made of healthy food choices. The functionality of a food is in its richness in bioactive naturally occurring compounds. This is particularly true for seed of hemp (*Cannabis sativa* L.), an eco-friendly and sustainable crop of recent renewed interest, which is a rich source of nutrients and non-nutrients.[1] In fact, its daily intake could provide nutritional and functional support for humans, as its oil contains a good n-6:n-3 essential fatty acids ratio (nearly 2.5:1 - 3:1) and its proteins are bearer of high nutritional benefits. Indeed, the massive abundance of these nutrients has masked for a long time the weak but not negligible presence of bioactives as phytocannabinoids and polyphenols.[2] In this context, seeds from different hemp cultivars (suitable for seed and/or dual-purpose - seed and fiber) underwent ultrasound assisted maceration using as extracting solvent *n*-hexane first and then ethanol, thus obtaining oil-like and alcoholic extracts. The first ones, metabolically profiled by ultrahigh-performance liquid chromatography (UHPLC) coupled with electrospray ionization (ESI) quadrupole/time-of-flight (QqTOF) mass spectrometer, mainly consisted of polyunsaturated fatty acids, but the sensitivity and selectivity of the MS method allowed also the identification of non-psychoactive phytocannabinoid acids differently oxygenated, whose relative amount largely differed based on hemp cultivars.
The chemical complexity of ethanol extracts was partially resolved only after their fractionation on SiO_2 column chromatography. Hydroxycinnamoyl amine conjugates (HAAs) and lignanamides were key constituents, beyond simple phenols and flavonoid compounds (prenylated and not). The obtainment of a polyphenol fraction, based on HAAs and their oxidative coupling derivatives, from one of the investigated hemp cultivar, was pursued and its scavenging capability was assessed by DPPH and ABTS methods. Cytotoxicity evaluation by means of MTT, SRB and LDH assays highlighted that it exerted a dose- and time-dependent anti-proliferative efficacy towards human keratinocyte HaCaT cells, hepatoblastoma HepG2 cells, epithelial colorectal adenocarcinoma Caco-2 and neuroblastoma SH-SY5Y cell line. In particular, upon treatment with fraction dose levels higher than 25 µg/mL clear cell morphological changes were observed as cells shrunk and became round.

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Phenolic acids from baru nuts target cell proliferation in a 3D cell model of colorectal cancer

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The Cerrado of Brazil produces the baru almond (*Dipteryx alata* Vog.), an edible seed from the fruit of the baru tree, which belongs to the legume species (*Fabaceae* family)[1]. Roasted baru nut is very consumed in Brazil where it is known for its high nutritional value and enjoyable taste similar to peanut. This study characterized the phytochemical composition of roasted baru nuts, focusing on free and bound phenolic compounds using an ultrasound-assisted methodology and analysis by high-performance liquid chromatography coupled to a mass spectrometer[2]. The antioxidant and electrochemical activity of the extracted fractions were evaluated[2]. Cell based assays using a human colorectal cancer cell line (HT29) were performed to explore the effect of baru nut extracts and isolated bioactive compounds in inhibiting cancer cell growth using monolayer cultures and a 3D cell model (cell spheroids)[3]. The phenolic compounds identified in free and hydrolyzed extracts were mainly isoferulic acid, p-coumaric acid, ellagic acid and gallic acid. Gallic acid and ellagic acid are electrochemically active under the conditions of analysis and this can be related to the antioxidant activity of the extracts. Results showed that both extracts of baru nut had the ability to impair cell proliferation in a dose-dependent manner in both monolayer and spheroid cultures (Figure 1). The free phenolic compounds extract of baru nut was more effective than the one obtained from the bound compounds in 3D cell model. Phenolic acids were pointed to be main compounds responsible for the anticancer potential of baru nut extracts.

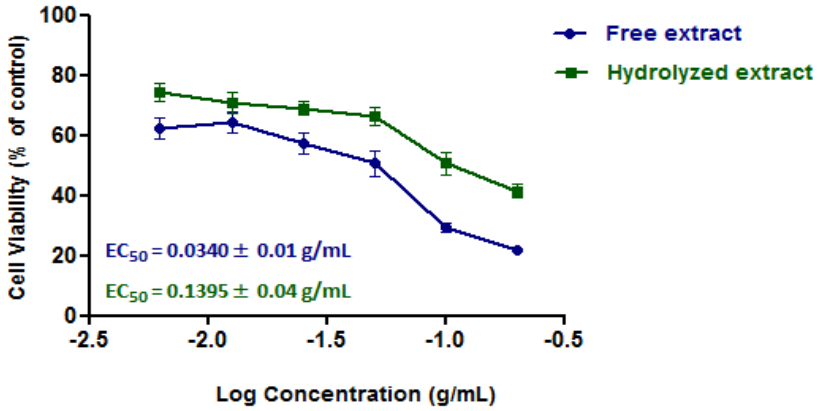

Fig.1. Free and hydrolyzed extracts of baru nut exerted antiproliferative effects in a dose-dependent manner after exposition for 72 h. Dose-response profile of the antiproliferative effect induced by both extracts in HT29 cell spheroids used as a 3D cell model of colorectal cancer (CRC). Results are means of three independent experiments performed in triplicate ± SD.

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Extraction optimization of phenolic compounds from *Fucus spiralis*

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Fucus spiralis has demonstrated a great antioxidant potential. However, many studies used different extraction methods and conditions. Thus, the aim of the present study was to evaluated differences between methods and search the better condition of extraction. In present study we had a green extraction purpose, so we used water and ethanol solvent. Response surface methodology was used to investigate the effect of extraction conditions including solvent:seaweed ratio (2:100–1:1), water: ethanol ratio (0:100-100:0), and extraction time (30–300 s) on the total phenolic compounds of brown seaweed (*Fucus spiralis*) extracts. Also was evaluated influence of extraction method including vortex, ultrasounds and sonicator. The optimum extraction parameters for maximum phenolic content were as follows: solvent:seaweed ratio, 1:2; water: ethanol ratio, 50:50; and extraction time, 300s on sonicator. The experiment results showed that the solvent:seaweed ratio and time was the most significant parameters for the extraction on sonicator. Under the above-mentioned conditions, the experimental total phenolic content and antioxidant activity value were 474.158 ± 19.751 mg GAE/g seaweed, of seaweed tested, which are well compatible with the predicted contents.

Table 1. Response surface methodology matrix and phenols results for different extraction conditions and different methods.

Time (s)	**Solvent:seaweed ratio** (g/100mL)	**Water: ethanol ratio** (quantity of water ml)	**Vortex** (mg GAE/g)	**Ultrasounds** (mg GAE/g)	**Sonicator** (mg GAE/g)
245,300	11,700	79,700	44,069	106,566	269,446
245,300	11,700	20,300	71,700	177,316	179,602
165,000	2,000	50,000	36,476	71,027	181,251
30,000	26,000	50,000	108,489	197,548	211,724
84,700	11,700	20,300	100,461	290,246	291,172
165,000	26,000	100,000	53,907	140,764	269,590
84,700	40,300	20,300	155,627	--	474,158
165,000	26,000	50,000	106,256	163,020	460,033
300,000	26,000	50,000	25,036	400,117	220,831
165,000	50,000	50,000	86,010	321,311	435,080
84,700	11,700	79,700	61,156	66,718	173,292
245,300	40,300	20,300	176,259	154,217	408,048
165,000	26,000	50,000	80,832	162,892	165,332
165,000	26,000	0,000	95,772	171,794	262,347
245,300	40,300	79,700	63,433	170,467	94,777
84,700	40,300	79,700	1,464	170,662	213,302

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Effect of Different Encapsulating Agent Combinations on Physicochemical Properties and Stability of Microcapsules Loaded with Phenolics of Plum (*Prunus salicina* Lindl.)

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Microencapsulation improves the bioavailability and stability of phenolic compounds, while encapsulating agents affect the quality of microcapsules. In order to improve the stability and application range of phenolics from plum (*Prunus salicina Lind*l.), microencapsulation was applied by using spray drying method. The present work evaluated the influence of maltodextrin/gum arabic (MD/GA), maltodextrin/gelatin (MD/GEL), maltodextrin/chitosan (MD/CHI) and maltodextrin/β-cyclodextrin/gum arabic (MD/β-CD/GA) on physicochemical properties and stability of microcapsules loaded with phenolics of plum. These four kinds of encapsulating agent combinations significantly improved the product yield and storage stability of the microcapsule powder, but there were differences among them in microencapsulated efficiency, microstructure and color. Using encapsulating agents markedly increased the glass transition temperature (Tg) of spray-dried powders, and Tg of the powder was positively correlated with its product yield. Furthermore, the powder prepared by MD/CHI had the best stability, and the retention rate of total phenol was 94.60% after storage at 25°C for 60 days. The powder prepared by MD/GA and MD/β-CD/GA preferably retains the original red color of plum, and can be used as natural nutrient red pigments in the food industry.

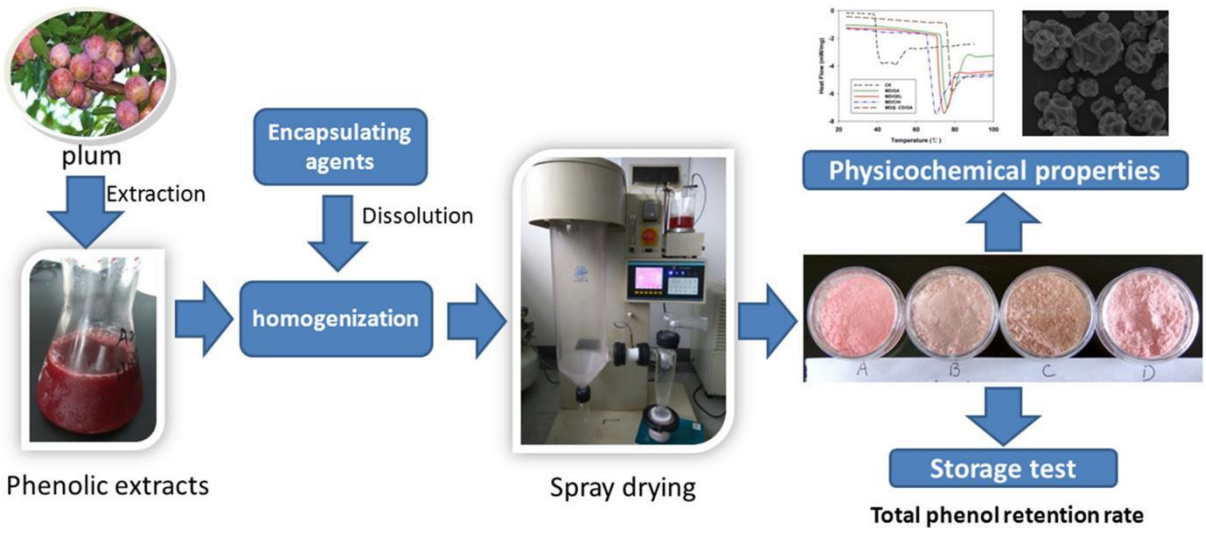

Fig.1. Schematic description about the microencapsulation of phenolic extract from plum by spray drying technique.

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Pineapple by-products integrated valorisation towards functional foods

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A high amount of industrial by-products are produced every day through processing of tons of fresh fruits. The processing generates a positive impact in the economy, but a negative impact on the environment, through the production of wastes that implies a loss of nutrients. During processing ca. 60% (w/w) of the pineapple fruit is lost, since usually only pineapple pulp is used for the production of products, so the main by-products generated are crowns (leaves), stems (core) and peels (Figure 1). Through green chemistry approach was possible to develop a fractionation of pineapple stems and peels, leading to the development of six pineapple new products (ingredients), two enzymatic high purity grade extracts, two pineapple liquid fractions with high antioxidant capacity and two pineapple flours with high content of soluble and insoluble dietary fiber. The enzyme fraction represented around 0.26% (w/w) of total fresh pineapple fresh weight for both by-products. Pineapple liquid fractions for stem by-product represented 68.5% (w/w) [4.8% (w/w) in dry basis] and for peel represented 57.7% (w/w) [17.3% (w/w) in dry basis]; pineapple solid fractions for stem represented 31.2% [3.1% (w/w) in dry basis] and for peel represented 42.2% [11.4% (w/w) in dry basis] of the total fresh pineapple weight. The total content of phenolic compounds has shown differences, the peels fractions showed lower content than the stems fractions, and the same tendency was found for the antioxidant capacities assays, as well as, the content total of vitamin C. Through HPLC analysis was possible to identify eight main phenolic compounds (for stem liquid fraction, while for peel liquid fraction seven were identified, and for both fractions was possible to quantify three compounds (chlorogenic, caffeic and ferulic acids). This data was corroborated by LC-ESI-UHR-QqTOF-MS where besides the 8 most representative where also identified new 11 compounds in small amounts. The fractionation by a green chemistry approach allowed the characterization and the study of different fractions of two pineapple by-products, leading to clear innovation by the development of six new ingredients. Therefore, this work has opened the opportunity to the market of new green products, as a result of by-products valorisation, not only for food industry, but also for nutraceutical and cosmetic industries.

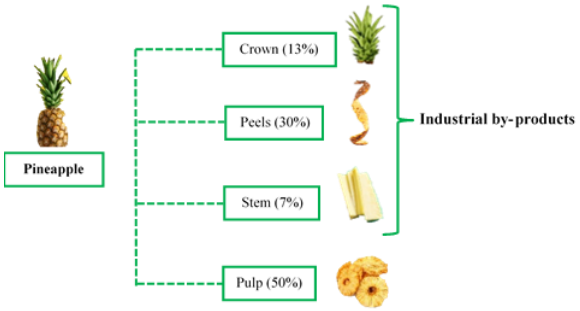

Fig.1. Flow chart of pineapple by-products identification in fruit processing industries.

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Functional features of two varieties of *Cucumis melo* L.: pulp and by-products

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Melon (*Cucumis melo* L.) is a very appreciated fruit, consumed all over the world, due to its excellent flavour qualities and nutritional composition, being an excellent source of bioactive compounds for humans [1]. Nevertheless, it contains large amounts of seeds and peel, which up to now are discarded. Food by-products are a major concern nowadays and their valorisation as food ingredients, for the development and formulation of new food products, is advisable and an emergent issue.

The aim of this study was to evaluate the nutritional composition and the vitamin C content of pulp and by-products (peel and seeds) of two different *Cucumis melo* L. varieties (Grand Prix and Soleares).

The *Cucumis melo* L. samples were collected, in 2019, from major supermarket chains in the Lisbon region (Portugal). Nutritional composition was determined according to Albuquerque et al. (2016) [2]. The energy value and available carbohydrates were calculated according to [3,4]. Separation and quantification of vitamin C (L-ascorbic acid) was performed by HPLC with diode array detection, using a Synergi™ Hydro-RP analytical column (150 x 4.6 mm I.D., 4.0 µm particle size) and UV detection was achieved at 245 nm [5].

From the obtained results, it is possible to verify that dietary fibre is the main constituent of the seeds, 46.5 g/100 g (Grand Prix) and 46.3 g/100 g (Soleares). Regarding to the total fat content of the seeds, Grand Prix presented higher content than Soleares, 28.5 and 22.9 g/100 g, respectively. The protein content of the seeds was 22.0 g/100 g for Grand Prix and 19.6 g/100 g for Soleares. The water is the main constituent (>90%) of the pulp and peel of the two varieties. The pulp and by-products did not present vitamin C content.

In summary, *Cucumis melo* L. Seeds can be considered a good source of dietary fibre, total fat and protein. On the other hand, *Cucumis melo* L. peel can also be considered a good source of dietary fibre. Melon by-products are a potential source of natural food ingredients that can be used to enrich and create novel foods with beneficial properties having in view health and well-being promotion.

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Functional properties of tomatoes in the aspect of inhibition of advanced glycation end products formation and activity of antiangiotensine and antiacetylocholinesterase

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Tomatoes are one of the most widely consumed vegetables around the world. They possess a high nutritional value as they are a rich source of many phytonutrients, including phenolic compounds. Diets rich in polyphenols are positively associated with a reduced risk of diabetes, hypertension or Alzheimer's disease [1,2,3,4,5].

The aim of our work was to investigate polyphenols profile and antioxidant capacity of extracts obtained from four different tomato varieties *i.e. Maliniak*, *Cerise*, *Black Prince* and *Lima*. Since functional properties are of a key significance to the nutritional quality of food products, the antiglycation (AGEs) effect as well as the extracts ability to inhibit the antiangiotensine (ACE) and antiacetylocholinesterase (AChE) activity were also studied. The antioxidant capacity was measured by photo-induced chemiluminescence (PCL) and ferric reducing antioxidant power (FRAP) assays, while the phenolic profile with HPLC-MS/MS. AGEs formation, ACE and AChE inhibitory activity were assessed by previously described methods [3,4,5].

The PCL values obtained ranged from 0.58 (*var. Maliniak*) to 2.60 (*var. Cerise*) µmol Trolox per g fresh weight (fw). The highest FRAP value was recorded for the extract of *Cerise* tomatoes (1.54±0.07 µmol Trolox/g fw), whereas the lowest for *var. Maliniak* (0.56±0.02 µmol Trolox/g fw). The dominant polyphenols identified in the extracts of *var*. *Cerise*, *Black Prince* and *Lima* tomatoes were rutin and quercetin. The results obtained indicated that the concentration of both of these flavonoids in the extracts analyzed was a key determinant for the inhibitory effect against AGEs formation, ACE and AChE activity. The extract of *Cerise* tomato with the highest concentration of rutin (23.01±0.11 µg/g fw) and quercetin (0.09±0.00 µg/g fw) was found to be most potent to inhibit (IC_{50}) the formation of AGEs and the AChE activity. On the other hand, all the tested tomato extracts manifested the ability to inhibit ACE activity, and the IC_{50} values obtained ranged from 2.26±0.00 mg/g fw (*Cerise*) to 3.05± 0.01 mg/m fw (*Black Prince*).

The results obtained indicated that the tomato variety had significantly determined the concentration of phytochemicals, the antioxidant potential of extracts, as well as the inhibitory effect against the formation of AGEs, and activity of AChE and ACE.

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Goat Probiotic Whey Cheese: Development and Nutritional Value

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There is an increasing awareness that a stable good health status is directly associated with dietary habits. This correlation has prompted a number of research studies and development efforts focused on functional foods. Accordingly, several products have reached the market, of which ca. 65% have been claimed as probiotic foods [1]. These foods include probiotic strains, belonging to the *Lactobacillus* and *Bifidobacterium* genera, which can prevent health disorders, and even improve health conditions via adequate colonization of the lower intestine [2]. Incorporation of probiotic bacteria has been successfully performed in several dairy matrices including whey cheese [2].

Whey cheese is a dairy product produced from ovine, bovine or caprine milk in the Mediterranean countries [3]. “Requeijão”, the Portuguese name for traditional whey cheese, is manufactured from raw or pasteurized skimmed milk through heating of whey at temperatures between 90 and 100 °C for 15–30 min, with or without the addition of 10-20% of ovine/caprine/bovine milk [3].

The objective of this work was to produce and characterize the nutritional composition of goat probiotic whey cheese incorporating a mix of two probiotic bacteria (*Lactobacillus rhamnosus* R11 and *Bifidobacterium animalis* subsp *lactis* BB12) and evaluate the stability of these two bacteria over 21 days of shelf-life under refrigerated conditions. Nutritional composition was determined according to AOAC methods for protein, lactose, moisture, fat and ash contents. Total fatty acid qualitative and quantitative profiles were determined according to Pimentel et al. [4]. The total amino acid content was determined according to Pripis-Nicolau et al. [5]. The probiotic whey cheese presented a high nutritional value with 11% (w/w) of proteins, 7% of lipids (w/w), 3% (w/w) of lactose, 2% (w/w) of minerals and 77% (w/w) of moisture. It is noteworthy, that the incorporated probiotic strains cell numbers remained stable or even increased slightly during the storage period: viable cell numbers of *Lactobacillus rhamnosus* R11–varied between 7.05 ±0.11 Log cfu/g (0 d) and 7.81±0.09 Log cfu/g (21 d) and those of *Bifidobacterium animalis* subsp *lactis* BB12 varied between 6.85 ±0.21 Log cfu/g (0d) and 7.86±0.14 Log cfu/g, coupled to a range of pH value between 6.1±0.03 at 0 days and 5.76 ±0.03 at 21 days.

In relation to the fatty acid profile, the most prevalent and stable compounds were capric acid (C10), myristic acid (C14), palmitic acid (C16), stearic (C18) and oleic acids (C18:1). Moreover, it is important to emphasize the presence of important omega-3 and omega-6 fatty acids like α-linolenic (C18:3), linoleic (C18:2) and arachidonic acids (C20:4). During the storage period the content in SFA (saturated fatty acids), MUFAs (monounsaturated fatty acids) and PUFAs (polyunsaturated fatty acids) acids varied between 7.1-10.3g/100g, 1.8-2.6g/100g and 0.2-0.3g/100g, respectively.In what concerns free amino acids, the most prevalent between 0 and 21 days were aspartic acid (4.94-7.66 mg/g of sample), glutamic acid (17.2-32.1 mg/g of sample), glutamine (8.65-10.9 mg/g of sample), arginine (19.4 -29.6 mg/g of sample), alanine (22.2 – 28.4 mg/g of sample) and valine (6.7-10.7 mg/g of sample).

Taking this into account, a new nutritionally-balanced whey cheese with a culture of *Lactobacillus rhamnosus* R11 and *Bifidobacterium animalis* subsp. *lactis* BB12 was successfully produced, enabling the survival of adequate viable cell numbers of the probiotic strains over 21 days refrigerated storage.

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Generation and characterisation of phenolic rich extracts from brewers' spent grain with hypotensive properties

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Brewers' spent grain (BSG) is a co-product of the brewing process which has previously been identified as a rich source of phenolic acids. With the exception of ferulic acid, few studies have evaluated the ability of these phenolic acids to inhibit angiotensin converting enzyme (ACE), a key enzyme in the renin-angiotensin system involved in blood pressure control. Enzymatic and alkaline approaches were employed to extract the phenolic components from BSG. The phenolic rich extracts obtained using the enzymatic approach had significantly lower ash content compared to those obtained as a result of the alkaline extraction. The phenolic content of the samples ranged between 0.87 ± 0.07 and 17.79 ± 0.56 mg gallic acid equivalents (GAE) g^{-1} BSG dry weight. Ferulic, p-coumaric and caffeic acid were identified in the extracts using reverse phase high pressure liquid chromatography (RP-HPLC). The phenolic rich fractions were characterised by their *in vitro* ACE inhibitory activity properties. Enzymatic treatment led to phenolic rich fractions with significantly lower ACE IC_{50} values (0.133 ± 0.032 to 0.351 ± 0.041 mg mL^{-1}) than those from alkaline extraction (ACE IC_{50} values of 0.528 ± 0.072 to 1.051 ± 0.107 mg mL^{-1}). The phenolic rich extract obtained using a combination of carbohydrase pre-treatments, i.e., with Shearzyme®, Ultraflo® and Depol® (1:1:1), displayed the highest ACE inhibitory potency (IC_{50}: 0.133 ± 0.032 mg mL^{-1}) and its hypotensive effects were studied in spontaneously hypertensive rats (SHR). Following ingestion of this extract (50 mg kg^{-1} body weight) a significant ($p < 0.05$) reduction in systolic blood pressure was observed 2 h post administration. The extract and the vasodilator drug Captopril® (10 mg kg^{-1} body weight) displayed similar reductions in systolic and diastolic blood pressure, and in heart rate and mean arterial pressure 6 h after oral gavage of the SHR. These results indicate that phenolic rich extracts from BSG may find applications as natural ingredients for the management of hypertension.

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In vitro investigation of the health promoting benefits from different food matrices: From extraction to characterization and *in vitro* evaluation

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Phenolic compounds are secondary metabolites produced by plants. They are constituents of several food matrices, like berries, fruits and leaves. Despite their wide distribution, the health effects of dietary phenolic compounds have come to the attention of nutritionists particularly in recent years. This interest was driven by epidemiologic data as well as *in vitro* and *in vivo* studies, suggesting that these compounds have a preventive effect in cancer, diabetes and cardiovascular diseases. Moreover, the antioxidant properties from food matrices also gained the attention of the pharmaceutical industry which looks for new ingredients for its formulations to fight against the described diseases.

The present study provided a comprehensive *in vitro* investigation into the health promoting benefits (antioxidant, antidiabetic and angiotensin-converting-enzyme (ACE)-inhibition) of different food matrices. For that, extraction strategies, such as µSPEed, QuEChERS and traditional extraction techniques such as solid and liquid-liquid extraction were performed (after previous optimization) to obtain a rich extract. Then, the total phenolic and flavonoid contents were determined, by a simplified and miniaturized methodology. The antioxidant capacity was achieved by DPPH miniaturized method, and the abilities to inhibit the angiotensin-converting-enzyme (ACE) and other digestive enzymes (α-amylase, α and β-glucosidase and lipase) were performed after the optimization of several parameters such as time of incubation and relation between the amount of enzyme/substrate/inhibitor.

The obtained results were very promising for classification of the tested food matrices according their health promoting benefits. Moreover the results allowed the selection of samples which have an increased potential in food chemistry as well in in the pharmaceutical industry.

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Preparation and Purification of Antioxidative Peptides Generated from Enzymatic Hydrolysates of Perilla Seed Meal

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Perilla frutescens is an oil crop with high nutritional value, whose seeds are rich in unsaturated fatty acids and high quality proteins. After the extract of oil from seeds, Perilla seed meal (PSM) is usually fed to livestock or discarded. The proteins left in PSM contain all essential amino acids and have great potential for development. In order to utilise PSM protein by-products, they were hydrolysed by different proteases to obtain antioxidative peptides. Taking the degree of hydrolysis (DH) and antioxidative activity as criteria, hydrolysis process study was carried out by single factor method and response surface method successively.The optimal conditions were as follows: Alcalase as the protease of hydrolysis, liquid-solid ratio of 22.32:1, enzyme concentration of 7.01%, hydrolysis temperature of 61.39℃ and time of 4h. Hydrolysate generated from PSM under the above conditions displayed a high degree of hydrolysis of 26.54% and high DPPH radical-scavenging activity.

This hydrolysate was fractionated into three parts by ultrafiltration through Millipore Amicon Ultra tubes with Molecular Weight Cut-Off (MWCO) of 10kDa and 3kDa in sequence. Fraction F_1(<3kDa), which exhibited the highest DPPH scavenging activity, was then fractionated by size exclusion chromatography on a Sephadex G-25 into three major fractions (P_1–P_3). Then fraction P_3, the highest DPPH scavenging activity among the three, was fractionated by reversed-phase high performance liquid chromatography (RP-HPLC). Four main fractions were isolated, and the fraction $P_{3\text{-}4}$ displayed the highest DPPH radical-scavenging activity (58.8 ± 1.07%; at 3 μg/ml) among these fractions. The results of this study suggest that PSM protein hydrolysates are good source of natural antioxidants. Further research would be conducted in order to identify the molecular masses and amino acids sequences of the purified peptides and their antioxidative activity in vivo.

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ORAL COMMUNICATIONS

Food Packaging

BIOFOODPACK - Biocomposite Packaging for Active Preservation of Food: the project and the progresses

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BIOFOODPACK (FIG. 1) is a M-era.NET project aiming to develop a sustainable biocomposite food packaging material to actively interact with foodstuffs, leading to improved food safety with minimal processing, reducing food loss and waste. Antimicrobial and antioxidant properties of natural resources are combined with different fillers to achieve water resistant materials with enhanced mechanical and gas barrier properties and electrically conductive for in-pack low temperature sterilization by pulsed electric fields.

University of Aveiro (Portugal) is the Coordinator of BIOFOODPACK and is the responsible for development of novel sustainable polysaccharide-based antimicrobial and antioxidant composites with optimized physicochemical properties. Wroclaw University of Science and Technology (Poland)-WRUT is in charge of studying the introduction of conductive species into both biopolymers and blends of biopolymers and synthetic polymers. The University of Minho (Portugal) tests applicability of the electrically conductive biocomposites in the sterilization of packaged food using PEF. The interaction of the packaging material with the foodstuffs using the typical pasteurization and the novel PEF processing will be studied under different environmental conditions. Migrations of fillers and its possible toxicity will be performed by Cyprus University of Technology (Cyprus). Three companies are involved: 1) MKF-E, a leading supplier of films, will enable the large-scale production of the packaging films; 2) SONAE, a market leader retailor will be involved on the testing of the biocomposite as packaging material at industrial environment, producing food samples packaged with the developed materials; and 3) Energy Pulse Systems, developing, producing and commercializing pulsed power modulators, will determine the technical requirements of the PEF equipment to process already packaged food.

One of the most important outcomes of this project is the establishment of strong synergies between partners that in a short-term period (3 years), leading to a biocomposite packaging active in food preservation, effective for in-packaged mild temperature sterilization and playing a role in the extension of food shelf-life. Technology Readiness Level will go from 1-2, up to 6 at the end of the project. In the long term (5 years) it is expected to turn the biocomposite into commercially available solution product(s).

Bio FoodPack
BIOCOMPOSITE PACKAGING
FOR ACTIVE PRESERVATION OF FOOD

Fig.1. BIOFOODPACK logo.

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Innovation in food packaging: a retailer's perspective

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Environmental and sustainability trends, together with consumers' awareness and shifting behaviours, are demanding a new approach to the future of our food systems [1] with a noticeable impact on food packaging, namely in what concerns plastics-based packaging.

Retailers are seen as key value-chain actors when it comes to rethinking current solutions and practices, related to packaging, and promoting new ones. Through the definition of challenges and requirements, and providing real world testbeds to foster validation of new developments made, food retailers can play an active role in driving innovation in food packaging.

Having recently signed Ellen McArthur's New Plastics Economy Global Commitment [2], Sonae MC, a leading Portuguese food retailer, is committed to eliminating unnecessary plastic items, innovating to help develop and test new packaging solutions (designed to be safely reused, recycled, or composted), and fostering circular economy principles applied also to packaging.

With the participation in R&D projects such as Biofoodpack (Biocomposite Packaging for Active Preservation of Food), YPack [3] (High Performance Polyhydroxyalkanoates Based Packaging To Minimise Food Waste) or Social Challenges [4] (Matching Social Challenges To Entrepreneurial Innovation), to name just a few, Sonae MC is actively co-developing and testing new packaging solutions that aim at extending products shelf-life and reducing food waste, whilst ensuring food safety and food properties.

Acknowledgments: The authors acknowledge the importance of the Biofoodpack and YPack projects, and respective project partners, in driving impactful innovation in food packaging

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GRAFOOD: 'Active GRAphene based FOOD packaging system for a modern society'

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The most investigated new preservation technologies for food are new packaging systems such as modified atmosphere packaging (MAP) and active packaging (AP), which use natural antimicrobials for biopreservation. AP is the incorporation of specific compounds into packaging systems that interact with the internal environment to maintain or increase product quality and shelf life of food. AP functions and technologies include moisture control, O2 scavengers or absorbers, CO2 controllers, odor controllers, antimicrobial and antioxidant agents, either natural or synthetic [1].
In this regard, packaging such as Ag/TiO2–SiO2-paper and Ag/N–TiO2-paper can find their applicability for extending the shelf life of white bread by 2 days as compared with the unmodified paper-package [2]. The literature reports the including of nanostructured TiO2 and Ag in paper, plastic, cellulose, chitosan, vegetable films in order to obtain efficient and safe food packages. Graphene oxide has shown high antibacterial activity against both Gram-negative and Gram-positive bacteria. The bacterial cellulose functionalized with graphene oxide showed a strong antimicrobial effect on *Saccharomyces cerevisiae* due to the effective direct contact between the nanofillers of the composites and the cell surfaces. The morphology of the nanocomposites has a great effect on physico-chemical properties and the interactions between the microorganism and the nanocomposites. By coating the graphene oxide with Ag nanoparticles, antimicrobial nanocomposites against the Gram negative bacteria (*Escherichia coli* and *Pseudomonous aeruginosa*) were synthesized. Probiotic lactobacilli (*L. rhamnosus* IMC 501® and *L. paracasei* IMC 502®) have a great potential to produce antimicrobial compounds that inhibit and control the microbial pathogen growth [3]. The aim of this work is to evaluate the new active packaging systems based on graphene oxide and probiotic microorganisms to preserve perishable food such as meat and cheese. The performances of new packaging systems have been evaluated by monitoring chemical, microbiological and sensorial parameters of wrapped food in comparison with commercial packaging.

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Development of clay-supported graphene materials for electrical conductive food packaging applications

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Food processing by pulsed electric fields (PEF) consists in the application of microseconds range high voltage pulses to food products to inactivate enzymes and microorganisms, while maintaining its sensorial and nutritional properties [1]. The food is processed before packaging, compromising the food safety due to post processing recontamination events [2]. The development of an electrical conductive food packaging appropriate to process food by PEF in-pack will overcome this limitation. Electrical conductive bionanocomposites are promising materials for this application, due to the non-toxic nature of both matrix and filler components. The synthesis of supported graphene materials using natural precursors such as sepiolite fibrous clay mineral and liquid caramel by pyrolysis in the 500-800 °C temperature range has been reported [3-5]. Herein, we produced caramel-sepiolite hybrids as graphene-like precursors trying to enhance the electrical conductivity by doping with MWCNT. It is here studied the pre-treatment of the caramel-sepiolite-MWCNT mixtures under hydrothermal conditions to promote the further carbonization of the caramel and to facilitate the next pyrolysis step necessary for the graphene-like materials generation. The evolution of materials structure after the treatments was studied by XRD, Raman spectroscopy and ^{13}C NMR. The morphology was evaluated by FE-SEM (**Fig. 1**). The thermal analysis by TGA and DSC was also performed. The specific surface area and the electrical conductivity were enhanced after the applied thermal treatments. Selected samples were incorporated into alginate biopolymer to develop electrical conductive bionanocomposites. Their mechanical properties and electrical conductivity in plane and through-plane was studied as a function of films thickness and carbon content. The sustainable character of these composites along with its mechanical properties and electrical conductivity make them promise materials for food packaging applications.

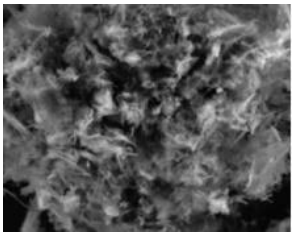

Fig.1. FE-SEM image of the sepiolite/caramel/MWCNT after hydrothermal treatment followed by a post-pyrolysis step at 800 ºC.

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Exploring the zinc oxide – reduced graphene oxide as an active composite in alginate films for food packaging application

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The main role of food packaging is to protect and preserve the product from external contaminations and physical damage, ensuring the maintenance of its quality and safety in the whole supply chain. The development of more sustainable food packaging materials with active properties is increasing in order to extend the shelf-life and/or retarding the deterioration of packaged food. In this context, active food packaging technologies involves the interaction between package or package components with food or internal gas atmosphere [1]. Zinc oxide nanoparticles have been considered feasible to incorporate in the food active packaging system and explored as an antimicrobial agent against bacteria. In addition, its high ultraviolet light absorption capacity avoids food deterioration by oxidation [2]. Furthermore, reduced graphene oxide (rGO) can provide electrical conductivity [3] to the food packaging material enabling its application in packaged food sterilization by the innovative pulse electric field methodology, where the food is minimally processed maintaining its original nutritional characteristics.

In this work, alginate biopolymer is used as matrix to prepare films reinforced with different concentrations of ZnO-rGO filler, previously synthesized by one step hydrothermal approach. The good dispersion of filler on alginate aqueous solution was obtained using sepiolite, a fibrous clay. The films were prepared by solvent casting and then immersed in a $CaCl_2$ solution, to turn the films more water resistant, and a glycerol solution as plasticizer. The films were characterized concerning their crystallographic characteristics, mechanical properties, water solubility, and surface hydrophobicity. The morphology was investigated using scanning electron microscopy (SEM) and the film cross-section showed that fibers of sepiolite are homogeneous inserted in the middle of ZnO-rGO and alginate matrix. The incorporation of ZnO-rGO and sepiolite to alginate matrix showed that the films are less rigid and resistant to fracture when compared with the alginate control film. The evaluation of electrical conductivity was also performed and showed that the increase loading of ZnO-rGO filler increases the electrical conductivity of alginate-based films. In addition, this filler adds to alginate matrix antioxidant activity.

Alginate films with the incorporation of ZnO-rGO allow to produce active films, with antioxidant properties and electrical conductivity, which can find application in food packaging field, particularly in the sterilization of packaged food by pulsed electric field methodology.

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Effect of MAP (high CO2%) on quality of fresh-cut non-climacteric vegetables in light of PCA with predictive biplots

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Modified packaging atmospheres (MAP) have been studied to establish appropriate conditions that maintain optimal quality parameters, such as, important nutritional compounds and organoleptic freshness [1,2]. Slowing of respiration rates while using appropriate packaging, temperatures and following safety and sanitation good practices, producers that sell directly to consumers or through distribution channels may gain time and reduce waste by adding shelf life days to their crops. The objective of this study was to compare the effect of an active MAP, rich in high % of CO_2 (40 and 45%) and reduced % of O_2 (10 and 15%), on bioactive compounds and freshness parameters (texture, organoleptic and microbiologic growth) of fresh-cut several vegetables such as cabbage, carrots and green beans, and green, red and yellow peppers. This study also aims at showing the advantage of a PCA analysis supported with biplots, which allows researchers to discuss results based on original values, whic is achieved by orthogonal projection of samples onto calibrated variable's axes [3].

Data mining using principle component analysis with predictive biplots, using Autobiplot.PCA function, allowed the selection of the most important parameters for sample discrimination (Fig.1). In contrast to common PCA analysis, with this method the selection of variables is automatic, and is only dependent on the magnitude of errors that are carried out reading biplots: if interpretation exceeds a given error, variables are automatically discarded from the final biplot solution. Also, based on a predetermined tolerance value, readings made in selected biplots axes are evaluated according to their accurateness in relation to original values, and outlier samples are automatically listed, avoiding common PCA problems, i.e., false interpretations and overestimations.

With these statistical tools, it was quickly seen that selected atmospheres generally promoted a quite stable environment over storage time. Texture and color where better preserved on peppers and carrots, although bioactivity in carrots presented greater losses compared to other samples. These results can be used as general guidelines for specialty crop producers involved in the short-term storage, packaging, and handling of a variety of vegetables commonly grown and minimally-processed

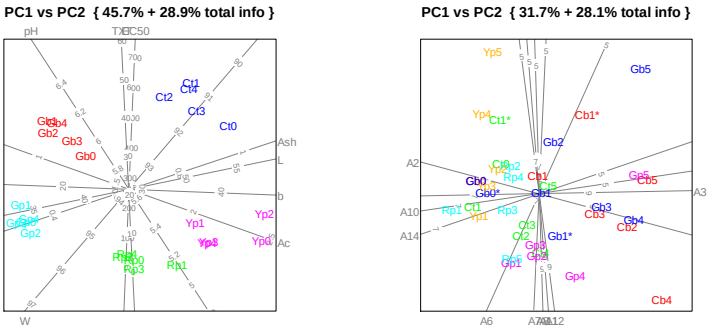

Fig. Predictive biplots made with Autobiplot.PCA function written in R, applied to physicochemical and sensory data.

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BioFoodPack Pulsed Electric Fields: in-package application for microbial inactivation for food products

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Nonthermal technologies for preservation extension of food products have been address in the last several years. These technologies present an alternative to conventional thermal processing, by delivering a safe product in terms of microbial contamination, without exposing it to thermal degradation.

One of these emerging technologies is Pulsed Electric Fields (PEF) treatment. This technology exposes the product to high electric fields in a controlled form (pulsed). PEF delivers high energy packages in a very short treatment time, allowing microbial inactivation with reduce thermal exposure of the product.

Nowadays, PEF technology is already being used at industrial scale for continuous processes for microbial inactivation of juices and other liquids. However, this process occurs before packaging and recontamination is still a possibility.

In the BioFoodPack project, we propose the development of a PEF application for packed products, which would allow the reduction of recontamination events and increase food safety for especially sensitive products (milk and dairy, fresh juices, fruit purees and others).

In this work, we present the approach to the development of a PEF application for packed products, highlighting the requirements and challenges to overcome, namely, PEF parameters and optimization, package material and electric characteristics (treatment chamber design).

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Agrofood byproducts as feedstocks for active food packaging materials

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Nowadays, the huge amount of landfilled plastic and agrofood wastes derived from the extensive trade in agrofood sector, the short shelf-life of fresh foodstuffs, and the plastic packaging materials give rise to severe environmental issues. Targeting to overcome these constraints, in this work, agrofood byproducts were used as feedstocks for the development of active biobased films, namely potato and coffee roasting byproducts. Starch/phenolics films using potato washing slurries and potato peels as a source of starch and phenolic compounds, respectively, and starch/coffee silverskin films were developed. The influence of phenolics and coffee silverskin amount on physicochemical, mechanical, and antioxidant properties of starch films was evaluated.

Both phenolic compounds and coffee silverskin improved the starch films flexibility, humidity resistance, and increased in more than 50% the antioxidant capacity of pristine starch films after 48 h of exposure to an $ABTS^{\bullet+}$ solution, respectively.

Therefore, the developed approaches are good examples of how circular economy brings benefits to industry, contributing for eliminating wastes by turning them into raw materials for the development of active food packaging materials.

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ORAL COMMUNICATIONS

Food Processing

Kinetics modeling and effect of drying temperature on new commercial grape 'Kyoho' skin: Evaluation for functional and antioxidant properties

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Grape skin (Kyoho), a by-product of processed grapes with potential food applications, was experimentally investigated for their drying behavior at different temperatures with five thin layer drying models. Moreover, we examined the effect of drying temperature on the bioactive potential of Kyoho skin. The experimental moisture ratio decreased with increasing drying temperature. The drying process of Kyoho skin was predicted by Page (303.15 K: R2 = 0.9734, 333.15 K: R2 = 0.9685) and Two-term (313.15 K: R2 = 0.9639, 323.15 K: R2 = 0.9737) models. Moisture diffusivity (Deff) ranged from 2.87 × 10-8 to 9.82 × 10-8 m2/s with an activation energy of 33.78 kJ/mol. Phenolics (0.37 to 0.23 mg GAE/g) and their antioxidant activities (DPPH•: 93.06 to 73.31 %) of Kyoho skin was significantly affected by drying temperature. These findings demonstrated the role of drying temperature on the bioactive potential to develop Kyoho skin value-added food products.

Keywords: Kyoho skin; hot air drying; thin layer models; retention capacity; antioxidant activity

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Stability of glucosinolate hydrolysis products and the identification of novel compounds in foods

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Vegetables of the Brassicaceae family such as cabbage (*B. oleracea* var. *capitata*) or rocket (*Eruca sativa*) are an integral part of our diet and source for glucosinolates. These compounds are precursors to cancer preventive isothiocyanates[1-2]. However, while in some vegetables mainly isothiocyanates are released, in cabbages epithionitriles are formed[3].

Due to thermal processing these compounds can degrade. As information on epithionitrile stability under typical food preparation conditions is scarce, we investigated the reactivity of selected epithionitriles and identified their main products. Moreover, the stability of Brassicaceae derived aliphatic ITC was investigated as well.

The epithionitrile from allyl glucosinolate (sinigrin), 1-cyano-2,3-epithiopropane (CETP) was very reactive compared to its higher homologs and an increase in pH drastically reduced its stability. The main product from CETP was 2-aminothiophene which was also found in cooked white cabbage sprouts, but also the dimer 1,4-dithiane-2,5-diacetonitrile was identified via NMR spectroscopy. From the higher homologous epithionitriles investigated several novel compounds were identified[4].
The stability of isothiocyanates was also highly dependent on their side-chain structure. Using NMR spectroscopy we could identify the main glucosinolate hydrolysis product in rocket to be 1,3-thiazepane-2-thione, a cyclic dithiocarbamate formed from 4-mercaptobutyl isothiocyanate[5].

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Gamma irradiation preserves nutritional and chemical composition of Agaricus bisporus Portobello

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Mushrooms are being increasingly produced and consumed, but their high perishability still represents a major drawback for commercial purposes. Besides the main effect of increasing the product shelf-life, the conservation technologies should be the most innocuous possible for the consumers. Among the current approaches, irradiation technologies seem to represent interesting alternatives [1, 2].

The present work reports the effects of gamma radiation (1, 2 and 5 kGy) and storage time (0, 4 and 8 days at 5 °C) on the nutritional (moisture, fat, proteins, ash, carbohydrates and energy) and chemical composition (free sugars, ergosterol, tocopherols, organic acids and fatty acids) of Agaricus bisporus Portobello fresh samples, andthe results were compared with control samples (0 kGy, non-irradiated). The irradiation was performed in a Co-60 experimental four sources chamber. The proximate composition was evaluated by AOAC official procedures. Free sugars, ergosterol and tocopherols were determined using high performance liquid chromatography coupled to a refraction index, UV and fluorescence detector, respectively; organic acids were determined using ultrafast liquid chromatography coupled to a diode array detector, while fatty acids were determined using a gas chromatography coupled to a flame ionization detector [3].

Irradiation dose did not exert any remarkably negative effect over the nutritional parameters of stored (up to 8 days) Portobello samples, specifically the irradiated samples at 5 kGy indicated a preserved nutritional profile with the highest levels of protein. It was possible to observe the maintenance of fructose, mannitol and trehalose contents in the irradiated samples, along storage time. All the applied doses also preserved the total organic acids (oxalic, quinic and malic acids) in relation to the control sample. A higher ergosterol level was found in samples irradiated at 2 kGy, while the highest percentages of C16:0 (8.6%) were found in samples treated with 5 kGy.

Gamma irradiation was effective in maintaining nutritional and chemical profiles throughout the assayed time intervals. Accordingly, this technology might represent an effective preservation approach for Portobello mushrooms.

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Effects of Drying Conditions in Low-temperature Microwave-assisted Drying on Bioactive Compounds and Antioxidant Activity of Dehydrated Bitter Melon Slices (*Momordica charantia* L.)

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Bitter melon (*Momordica charantia* L.) is a highly nutritious food for culinary and therapeutic purposes due to its bio-active compounds (Tan et al., 2016). Dried bitter melon could be used as food or applied to produce pharmaceutical products. Removing moisture from materials was often used by drying techniques such as hot-air, vacuum, freeze, infrared, microwave drying, etc. Drying at low-temperature was considered to dehydrate materials with high biological activity. However, the main drawback in this technology is low-driving force for moisture removal. In studies of drying technology, microwave or microwave-assisted drying always reduces significantly the processing time based on volumetric heating mechanism and improved the quality of dried food products than conventional drying techniques (Kumar et al., 2014). Therefore, the combination of low-temperature convective drying and microwave radiation has been proposed to dehydrate vegetables containing high bio-active compounds such as bitter melon. Our study was conducted to evaluate the influence of microwave powers (150, 300, and 450 W), drying temperatures (20, 25, and 30 ^{o}C), air velocities (1.2, 1.9 and 2.6 m/s), and sample weights (50, 100, 150 and 200 g) on moisture content change, nutrient levels (vitamin C and total phenolics), and antioxidant capacities (DPPH and FRAP assays). The obtained results showed that all of investigated factors affected on moisture removal rate. The larger the microwave power or the temperature is, the shorter drying time is. On the contrary, the increase of air velocity or sample weight increased drying time. There are two groups of investigated factors which affected significantly on levels of nutrient (see fig.1): (i) microwave power and drying temperature and (ii) air velocity and sample weight. For the first group, the retention of nutrients after drying process would be dependent on the rate of degradation reaction and time. For the second group, the increase of air velocity or sample weight prolonged the drying time causing more loss of nutrient levels. Effects of drying conditions on antioxidant capacities were similar to change of nutrient levels. It was found that DPPH radical scavenging ability had positive correlation with total phenolics content, but, the ferric reducing antioxidant power was related to the presence of reductants including phenolic compounds, vitamin C and other phytochemicals in bitter melons. The appropriate drying conditions that reduced the drying time, preserved nutrient contents and antioxidant capacity were microwave power of 300 W, drying temperature of 30 °C, air velocity of 1.2 m/s and sample weight of 100 g. For further study, kinetics of moisture removal or nutrient degradation during drying will be determined to control operation. Besides, it is necessary to perform the study on pre-treatment techniques for inactivating enzymes which are not impacted by microwave radiation at low-temperature.

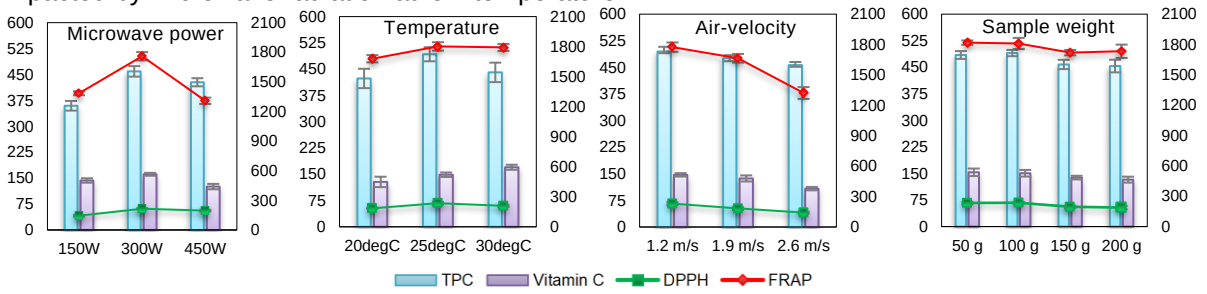

Fig.1. Changes of nutrient levels and antioxidant capacity at different drying conditions.
Notes: Primary vertical axis is nutrient levels (mg/100 g dry basis) and Secondary vertical axis is antioxidant capacity (mg TE/100 g dry basis

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Dicarbonyl Scavenging by Creatine in Food and *in vivo*

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Dicarbonyl compounds such as methylglyoxal and 3-desoxyglucosone (3-DG) are formed from sugars during food processing or in vivo from glycolysis by-products. They play an important role in food as color and odor precursors. Because of their high reactivity towards protein and their increased concentrations in blood of patients with diabetes, their possible pathophysiological role for metabolic diseases and aging is discussed.[1] Recently it was shown that creatine reacts rapidly with methylglyoxal under physiological conditions to form methylglyoxal-derived hydroimidazolone of creatine (MG-HCr, see Fig. 1). [2]
It was the aim of this study to investigate the reaction of creatine with dicarbonyl compounds in food, the metabolic transit of the reaction products and the *in vivo* formation of the reaction compounds.

From incubation mixtures consisting of 3-DG and creatine, a new hydroimidazolone of creatine, namely N-(4-butyl-1,2,3-triol-5-oxo-1-imidazolin-2-yl)sarcosine (3-DG-HCr, see Fig. 1), was isolated and characterized via NMR spectroscopy.[3] Meat and fish products were analyzed after extraction and protein precipitation, while urine samples were analysed after centrifugation and protein precipitation. MG-HCr and 3-DG-HCr were quantitated using isotopically labelled standard material and HPLC-MS/MS analysis.

Whereas samples of raw fish and meat contained only trace amounts of the hydroimidazolones, concentrations of MG-HCr (up to 28 mg/kg) and 3-DG-HCr (up to 15 mg/kg) increased during roasting.[3] The concentrations were dependent on food processing such as heat treatment and smoking. The derivatization rate of creatine as MG-HCr and 3-DG-HCr was higher than of lysine and arginine with advanced glycation endproducts. This demonstrates the 1,2-dicarbonyl scavenging properties of creatine in meat. Creatine and its adducts could also be quantitated in urine samples with concentrations dependent on the diet of the subject.

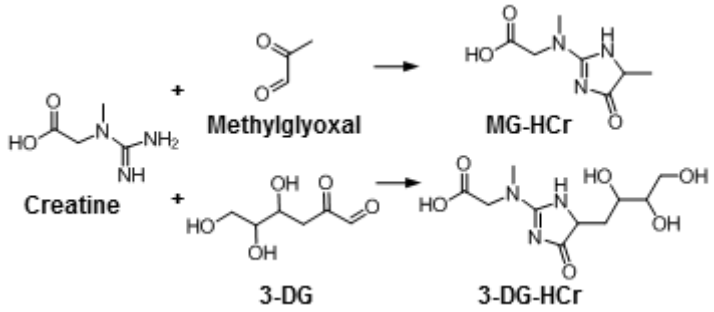

Fig. 1. Formation of MG-HCr and 3-DG-HCr

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Shiitake mushroom (*Lentinola edodes*) spread creams

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This work is part of Agrio et Emulsio project (POCI-01-0145-FEDER-023583), the main goal is formulating and design an innovative food emulsion based on processed *Shiitake* mushroom (*Lentinula edodes*), through sustainable methodologies with potential application in certain markets such as gourmet, diet and vegan [1].

Shiitake mushroom is a fungus of the *phylum Basidiomycota* and *Lentinus* gender. It is the second most cultivated edible mushroom in the world, currently accounting for around 25% of world production of edible mushrooms. Its importance nowadays, due to lifestyles and habits from Asian countries. It is considered a high-quality food with high content of protein, vitamins and minerals and low content in calories and fat [2].

An emulsion is a multiphase system consisting of two immiscible phases, one aqueous phase and a lipid phase, in which one phase is dispersed in another in the form of spherical drops. System stability depends on the membrane that holds the drops and varies over time. Spreads creams are water-in-oil emulsions which lipid phase is a mixture of vegetable oils and / or oils and animal fats, containing natural colorants, stabilizers, emulsifiers, flavourings, antioxidants, lecithin and liposoluble vitamins. The aqueous phase comprises skimmed milk proteins, and small amounts of other ingredients such as salt, preservatives, thickeners and water-soluble vitamins [3].

The methodology involved the experimental technological development. There were performed microbiological assays, proximal and physicochemical and sensory analysis. The aqueous phases were pasteurized for obtaining ideal binomial time / temperature confirmed by microbiological analysis.

Two final prototypes were selected, one of them vegan with aqueous phase of vegetal origin, and another lacto-vegetarian with aqueous phase of animal origin, whey protein concentrate of goat's milk. After this the prototypes were produced for stability tests, as well as physicochemical, proximal, rheological and organoleptic characterization.

Microbiological stability tests, proximal and physicochemical analysis, as well as food pairing and food design tests are carried out.

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Potential browning precursors within uronic acid reaction-systems

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Investigations in the past show the high browning potential during caramelization of sugar acids especially in comparison to reducing sugars[1,2]. The heat treatment of aqueous model-systems of D-GalA for example show a ten times higher browning compared to that of D-Gal or D-Glc.

The ring opening velocity may play an important role for understanding the drastic differences in the reaction speeds between uronic acids and reducing sugars. Polarimetric measurements show that the speed of mutarotation of D-GalA exceeds that of D-Gal by nearly 4.5 times. To examine whether the high reactivity of D-GalA goes back to its carboxylic functionality, formic acid was mixed in equal concentrations with D-Gal. The mutarotation rate constant was measured again and an enhancement of a factor of only 1.7 was registered.

But not only the ring opening velocity differs between the two carbohydrate structures. One other factor influencing the degradation reactions is decarboxylation of the uronic acid. Experiments measuring the concentration of CO_2 during a time series of heated D-GalA at 60 °C show a steady increase. Already after 2 hours the amount starts to rise, correlating with a decrease of the D-GalA concentration. After 48 hours approximately 6 % of degraded D-GalA have release CO_2. The main degradation reactions postulated for the release of CO_2 leads to α-ketoglutardialdehyde which is responsible for the quick formation of several chromophoric substances.

α-ketoglutardialdehyde seems to be one of the central degradation products, beeing a precursor moelcule of substances such as reductic acid and furfural. Next to these already very well known degradation products GC-MS investigations of aquatic model-systems of D-GalA at 130 °C show the formation of 2,3-dihydroxybenzaldehyde, catechol[3] and 3,8-dihydroxy-2-methylchromone[3], that after several experiments also seem to derive from α-ketoglutaraldehyde as intermediate product. Model-systems of 2,3-dihydroxybenzaldehyde already show an intense colour formation after 120 minutes. These results indicate that the formation of chromophoric substances within uronic acid model-systems derive from two different pathways. One leading from the formation of caramelization reactions typical for sugar degradation and the other one resulting from oxidative polyphenolic coupling reactions that do not take place within model systems of reducing sugars. Furthermore within D-GalA model-systems the two pathways seem to combine as ESI-MS measurements of model systems of norfuraneol and 2,3-dihydroxybenzaldehyde show polymerization products originating from condensation reactions between these two compounds.

Deeper insights into the reaction pathways of uronic acids are necessary and very important as they have an influence on the industrial production of fruit juices and may lead to unraveled color formations.

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Monitoring physicochemical and sensory attributes during debittering of stoned green olives

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Stoned green olives are traditional table olives produced in the Northeast of Portugal. They are highly appreciated due to their organoleptic characteristics and levels of complex phenols endowed with strong antioxidant activity, having a significant agro-economic relevance. During the natural debittering process, stoned olives are immersed in water, which is changed (each 1-2 days), leading to a reduction of the initial bitterness and increase of sweetness, turning these green olive edible [1]. Monitoring total phenols contents, the bitterness index as well as the basic sensorial attributes through this washing stage is very important. In this work, the debittering process of 110 samples of stoned green olives (*cvs.* Cobrançosa and Negrinha de Freixo), was monitoring during 20 days. After each debittering-washing time-period (water changed each 2-days) bitter, pungent and sweet intensities sensations were assessed by trained panelists. Furthermore, the bitterness index and total phenols contents were also evaluated spectrophotometrically. Figure 1 shows the trends observed for the sensory attributes and chemical parameters along the debittering-washing process. From the boxplots (Figure 1), it is clear that bitterness index and total phenols contents significantly decreased with the time, which could be directly related to the observed decrease of the bitter taste intensity (for both olive cultivars) while the decrease of the pungent sensation could be related with the decrease of the total phenols contents, mainly influenced by the increase of the sweet sensation. Furthermore, as can be inferred, the initial bitterness and pungency intensities of *cv*. Cobrançosa olives were higher than those from *cv.* Negrinha de Freixo. Nevertheless, after 16-20 days of debittering-washing steps, the bitter and pungent intensities perceived by the trained panelists, for both olive cultivars, were of the same order of magnitude, showing that the debittering procedure adopted was successful and technologically consistent.

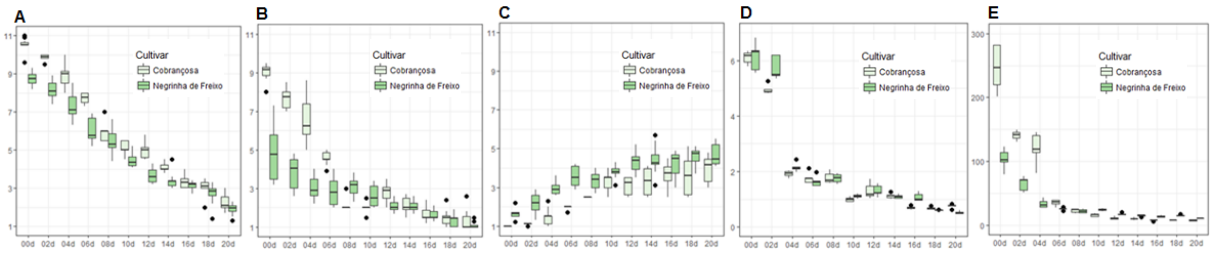

Figure 1. Boxplots showing the time evolution of **(A)** bitter, **(B)** pungent and **(C)** sweet as well as of **(D)** bitterness index (g Oleuropein/ kg) and **(E)** total phenols contents (g GAE/ kg) of the stoned green olives (*cvs.* Cobrançosa and Negrinha de Freixo) during the 20 days of debittering-washing process.

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Impact of low temperature on interactions and cohesiveness of casein micelles dispersions

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Caseins are present in the milk in form of casein micelles – porous, deformable and compressible colloidal particles [1, 2]. Understanding of interactions between the casein micelles and properties of concentrated casein micelles dispersions (e.g., compressibility, concentration of gel formation, gel cohesion) is important for optimization of milk filtration because appearance of the gel on filtration membrane surface reduces the filtrate flow. Bouchoux et al. [3-5] demonstrated that concentrated dispersions of casein micelles can be obtained and their properties can be characterized with the help of osmotic stress and swelling-redispersion experiments. It was found that casein micelles dispersions turn from the liquid state into the solid-like gel state at the micelles concentration of ≈ 180 g·L–1 [3, 4] and that cohesiveness of obtained gels strongly depends on their concentration [5]. These findings allowed to introduce the maximal critical casein micelles concentration on the membrane surface, and to define milk filtration conditions that do not lead to severe membrane fouling [6].

In previous experiments of Bouchoux and co-workers [3-6], casein micelles were characterized at the temperature of 20°C. However, there is an increasing interest to milk filtration at low temperature (≈8-12°C). Also, it is known that casein micelles have temperature-responsive porosity and chemical composition in diluted dispersions [1, 2]. Therefore, it is necessary to elucidate the influence of the temperature on compression, structure formation and swelling-redispersion of concentrated dispersions of casein micelles and to relate it with optimal filtration conditions.

The aim of the current work was to analyse the impact of temperature on interactions and cohesion within concentrated casein micelles dispersions. The experiments were performed at 7°C and 20°C. Casein micelles dispersions with different casein micelles concentrations (from ≈120 to ≈700 g·L–1) were obtained and casein micelles compressibility was characterized via osmotic stress experiments (from 0.05 to 13 bar). The cohesiveness of obtained gels was characterized via swelling-re-dispersion experiments: gel is introduced in milk solvent, then hydration and casein micelle re-dispersion rate are followed in function of swelling-re-dispersion duration (from 4 to 240 h). In order to distinguish the influence of the temperature on the gel structure and swelling kinetics, each gel sample was re-dispersed at two different temperatures (7°C and 20°C).

It was observed that compressibility (concentration attained at given osmotic pressure) of casein micelles increases with the temperature increasing from 7°C to 20°C. This can be explained by the reduction of hydrophobic bonds at lower temperature [2]. It was found that swelling of casein micelles gels at 7°C results in lower redispersion rate as compared to the swelling at 20°C regardless of the gel formation temperature. This can be partially explained by higher thermal motion at 20°C. However, it was also observed that casein micelles gels obtained at 7°C are more cohesive than those prepared at 20°C, since for high osmotic pressures, gels are not at all re-dispersed at 7°C, while they are almost fully re-dispersed, if prepared at 20°C. This can imply that more rigorous filtration conditions should be applied during the low temperature milk filtration.

Acknowledgments: We thank P. Bacchin for interesting discussions.

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Bioaccessible of D-*chiro*-inositol from water biscuits formulated from common buckwheat flours fermented by lactic acid bacteria or fungi

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D-*chiro*-inositol (DCI) is an active compound in common buckwheat (*Fagopyrum esculentum* Moench) with insulin-like bioactivity [1, 2, 3].

In this study, the bioaccessibility of DCI from water biscuits formulated from fermented raw and roasted common buckwheat flours was studied. The liquid-state fermentation (LSF) by select lactic acid bacteria (LAB) or fungi *Rhizopus oligosporus* 2740 was performed.

The study showed that LSF, baking and digestion (in *in vitro* conditions) significantly affected the DCI content in fermented flours, biscuits and the digestible matter of buckwheat water biscuits. LSF by *L. salivarius* AWH or *R. oligosporus* 2740 significantly enhanced the DCI content, whereas most of the applied LAB reduced DCI in the fermented flours. Baking at 220°C for 30 min significantly enhanced the DCI level; however, no correlation was found between the DCI content in fermented flours and the biscuits prepared from them. The potential bioaccessibility of DCI from water biscuits was lower than 1, thus indicating its low bioaccessibility. It can be concluded that select LAB and *R. oligosporus* 2740 applied for LSF, the heat treatment and physical structure of biscuits were mainly responsible for the potential bioaccessibility of DCI. These factors should be taken into account when DCI-enhanced water buckwheat biscuits are prepared.

Acknowledgment
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Thermal deterioration of ω-3 and ω-6 fatty acids under food processing conditions

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The role of nutrition in healthy aging plays an important role in our lives. There is an increasing desire for foods which have a health-promoting and disease-preventing effect. The attention of consumers is brought to the ratio of unsaturated ω-3 and ω-6 fatty acids (PUFAs) within food. Therefore, rapeseed oil is of great interest because it has a higher content of linolenic acid in comparison to other vegetable oils.
In conjunction with the research project (NutriAct), we are investigating the oxidative stability of rapeseed oil in various food matrices and under different processing conditions.
Unsaturated fatty acids rapidly degrade to volatile compounds. Hence, the stability of oil in food systems must be investigated, especially if food is enriched with ω-3 and ω-6 fatty acids, so that the health promoting effects remain. The degradation products formed by lipid oxidation are of interest because they decisively influence the quality of food. By using various analytical methods, the oxidative deterioration can be observed. For example, the volatile compounds are analysed by using GC-MS and the technique of Headspace. The rancidity of rapeseed oil is characterised by certain marker substances: Hexanal, 2-Hexenal, 2,4-Heptadienal, among others.

Due to the complexity of lipid oxidation, there are several factors that can affect lipid oxidation. In addition to the availability of oxygen, the heating temperature and heating time are the most important external influencing parameters. Among the internal factors that influence the progress of lipid oxidation are the concentration of free fatty acids and antioxidants, the degree of saturation of a fatty acid and the matrix of the food, especially the content of water.

Simultaneously to the compounds that result from lipid oxidation, thermal degradation products of sugars are also formed during the processing of foods. As part of our research, the influence of carbohydrates and their degradation products on lipid oxidation is investigated. The complexity of food matrix and the interaction between different degradation products of food compounds plays an important role in food quality and food spoilage.

Furthermore, the aim of this study is to ensure the highest possible oxidative stability of the essential ω-3 and ω-6 fatty acids during the production, storage and treatment of foods as well as to characterise the chemical interactions of degradation products of lipid oxidation with other food ingredients.

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Protein oxidation in food: Focus on individual structures

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Protein oxidation is widely described *in vivo* and is linked to “oxidative stress” [1]. This term denotes an imbalance between the occurrence of reactive oxygen species (e.g., hydrogen peroxide, hydroxide and superoxide radicals) and the capacity of antioxidant enzyme systems (e.g., catalase, superoxide dismutase) to quench these species. During food processing and storage, food proteins are also subject to oxidative degradation reactions that have a significant impact on food quality. In particular, essential amino acids such as methionine and tryptophan can easily be oxidized [2]. Methionine oxidation is associated with the light-struck off-flavor of milk. Protein oxidation in food is sometimes assessed by measuring “protein carbonylation” through reaction with 2,4-dinitrophenylhydrazine (DNPH), however, this method cannot give a reliable picture on protein oxidation in food, because structures such as methionine sulfoxide and oxidation products of tyrosine and tryptophan do not react.

In the first part of the present work, methionine oxidation was analysed in milk products by HILIC-HPLC-MS/MS. Milk proteins were isolated and hydrolysed enzymatically in the absence of oxygen. An isotopically modified methionine probe was added in order to record artificial methionine oxidation during hydrolysis. In UHT and evaporated milks, up to 8% of methionine was oxidized, whereas up to 33% of methionine was oxidized in milk drinks containing added cocoa or coffee components. The concentrations of methionine sulfoxide are far above the concentrations of protein carbonyls measured in milk products by the DNPH method [3]. In a model experiment, the influence of food constituents on methionine oxidation was assessed by HPLC-UV using benzoyl methionine as a substrate. When heated at 80 °C for 6 h, this compound was strongly oxidized to the sulfoxide in the presence of catechin (13%), caffeic acid (20%), and gallic acid (70%), but not in the absence of these additives.

In the second part, Hypercarb-HPLC-MS/MS was used to explore further prominent protein oxidation products in different foodstuffs after acid hydrolysis. 3,4-Dihydroxyphenylalanine (DOPA) was detected in bakery and milk products, 3-nitrotyrosine and aminoadipic acid in meat products, and dityrosine in milk-powder based sweets.

In the third part, oxidation-induced protein crosslinking involving tyrosine residues was investigated. In mixtures containing tyrosine and tryptophan that were exposed to a free radical-generating system, a new substance emerged in addition to the known dimerization products of tyrosine and tryptophan. The formation of a heterodimer between tyrosine and tryptophan as well as its spectroscopic characterization is shown for the first time. The compound was also detected during oxidation of model proteins.

We conclude that methionine oxidation is an important chemical deterioration pathway during protein oxidation in food. Compounds generally regarded as antioxidants may boost methionine oxidation. The use of the DNPH method for assessing protein oxidation in food is misleading due to the abundance of structures that do not react in the assay. Lastly, protein oxidation still holds many new individual compounds, whose role for food structure and safety needs to be explored in the future.

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Effect of drying methods on the properties of mixtures of aromatic plants for gastronomy using different encapsulated agents

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Excessive salt intake has a strong impact as a risk factor for cardiovascular diseases, with prevalence and high impact in Portugal and around the world. Currently there is extensive scientific evidence supporting the need for salt reduction in food. At the same time, concrete strategies to reduce the use of salt in food establishments are lacking.
The aim of this study was the development and application of microencapsulation of aromas and flavours from aromatic plants and spices to use in gastronomy.

Mixtures of aromatic plants and spices, powdered, were created. From these mixtures we developed oleoresins that were microencapsulated, with different encapsulates (starch, alginate, maltodextrin, inulin, arabic gum), to protect and increase aroma and flavor stability.

Maltodextrin and inulin presented the higher yield on spray drying and maintained aromatic characteristics closest to the seasoning with salt. Due to contend in dietetic fibber inulin was selected to develop the final microencapsulation using spray drying and lyophilisation processes. In the culinary preparations the salt used was replaced from 50% to 80% by the microencapsulate.

The analyses of microencapsulates were performed by HPLC-PDA, LC-MS, and sensorial evaluation by the following parameters: color, texture, aroma, taste and perception of salt.

HPLC-PDA results showed that lyophilisation process presented high stability to the compounds of the plant mixture relatively to the spray drier process. Results of the sensorial evaluation evidenced good acceptance for all parameters, in which salt perception, flavor and hedonic evaluation stand out.

In addition inulin encapsulates obtained by the two process presented high stability to use in culinary preparation at cooking temperature. However the spherical and dry formulation obtained by the lyophilisation process allowed simulating the use of common salt, combined with its total dissolution. It can be concluded that the products can be used as a concrete strategy for salt reduction in public catering and within individual context. The taste and aroma of the products allows its use either in a gradual reduction or for total salt elimination.

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Repitching impact on sugars and amino acids uptake by a lager yeast

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The object of this study is a lager beer, named Coral, which is a commercial beer produced at Madeira Island, namely at the local brewery *Empresa de Cervejas da Madeira*.

The reuse of the same yeast culture in consecutive fermentations is a procedure usually applied in brewing industry, which can generally vary between 7 and 20 times. Serial repitching as also the yeast management between fermentations have an important impact on fermentation performance. Therefore, in order to understand yeast efficiency, the wort fermentation ability should be evaluated [1,2,3]. In this sense, the evolution of wort and beer metabolites are very informative. Generally monosaccharides (glucose and fructose) are first metabolized by yeasts, following disaccharides (maltose), and trisaccharides (maltotriose), according to yeast preference. Also, amino acids uptake by yeast follows a preferable order affecting the rate of its consumption, which can be categorized in 4 groups [3].

The main goal of this study was to evaluate the uptake of fermentable sugars and amino acids through different generations of the yeast biomass. Several fermentation batches, three of each generation and produced from identical wort batches, were followed at the brewery and samples collected during 12 days of fermentation and analysed.

A reversed-phase high performance liquid chromatography technique was applied for the separation of individual amino acids with a pre-column derivatization methodology and the detection through a fluorescence detector. Sugars were detected through a refractive index detector.

Regarding sugars, results showed that yeast starts metabolizing glucose and fructose, following maltose and maltotriose. As for individual amino acids, there are slight differences in terms of the uptake preference showed by yeast, being the majority categorized in accordance to the literature. Also, results showed a faster consumption of sugars and amino acids through the 1st generation of yeast when compared to others, which means a better fermentation performance. The obtained data indicate that the conditions on which the yeast culture is stored for the next reuse are very important and may limit its efficiency in the next fermentation.

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MAILLARD induced color formation based on intermediates with activated methylene groups

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The degradation of carbohydrates in presence of amino compounds during processing and storage of food has an important impact on its flavour, taste, texture, color, and oxidative stability.[1,2] All these properties are caused by complex mixtures of MAILLARD intermediates and end products. The formation mechanisms and structures of the most important intermediates, such as 1,2-dicarbonyl compounds, acids, aldehydes, furans, furanones, pyranones, pyrrols, pyrazines, and early condensation products are well described in literature. On the other hand, reactions leading to colored high molecular MAILLARD reaction end products, also known as melanoidins, are studied in less detail.

Recently, we investigated the formation of colorants from the MAILLARD intermediates 3-deoxyglucosone (3-DG) and norfuraneol. Both compounds contain activated methylene groups that can easily undergo aldol reactions with carbonyls resulting in a high browning potential especially at elevated temperatures. Whereas 3-DG can form a kind of homopolymer,[3,4] the formation of high molecular weight colorants from norfuraneol is greatly increased by the involvement of additional carbonyl compounds.

To compare the reactivity of norfuraneol (**1** in Fig. 1) in presence of typical MAILLARD intermediates with carbonyl functions (**2** in Fig. 1) equimolar mixtures were heated at 130 °C for up to 300 min in aqueous solution (20 mM, pH 5) and for up to 120 min under solvent free conditions. The absorbance at 420 nm of samples containing norfuraneol without addition of a second compound was slightly increased when typical MAILLARD cleavage products like acetaldehyde, glycolaldehyde, and acetol were added. These aldehydes yielded mainly aldol addition products with norfuraneol (**3** in Fig. 1) that could not form oligomers in further reactions resulting in a limited color formation.

The addition of heterocyclic compounds such as furfural, hydroxymethylfurfural (HMF), and pyrrolaldehyd lead to a 4-fold or even higher increase in absorbance. The equimolar condensation products (**4** in Fig. 1) could be found as intermediates in trace amounts in the aqueous systems and as main products in the dry systems. In addition, organic extracts of both systems were analyzed with high resolution mass spectrometry (HRMS) and MS^n experiments to elucidate the structure of the colorants. Polymers of up to 15 units formed from alternating steps of aldol condensation and MICHEAL addition were identified and revealed a possible reaction mechanism leading to high molecular weight compounds based on heterocyclic MAILLARD intermediates.

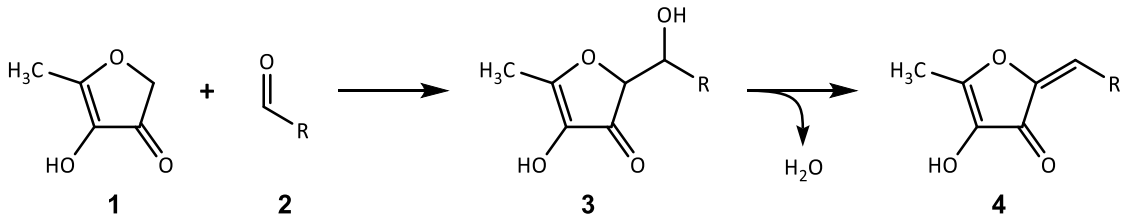

Fig.1. Aldol condensation reaction of norfuraneol **1** and an aldehyde **2**.[5]

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Broccoli by-products as ingredients rich in bioactive compounds after microwave assisted dehydration

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In the broccoli frozen-food industry, stalks, leaves, and inflorescences remains account for 45% of the initial broccoli heads sharing their nutritional value and bioactive compounds [1]. Their use as an ingredient in the design of functional foods is a promising valorisation strategy. However, due to their high moisture content, the valorisation of broccoli by-products as food ingredients requires stabilization to inhibit enzymes, prevent microbial growth, and degradation of the product [2]. Moreover, the selection of processing techniques should consider the impact on bioactive compounds.

Therefore, in this study, a new technology based on the application of microwave hydrodiffusion and gravity (MHG) was used to dehydrate broccoli by-products and simultaneously recover the water-soluble diffused compounds [3]. Moreover, MHG dehydration impact on pigments (carotenoids and chlorophylls), phenolic compounds, glucosinolates, protein, and carbohydrates was evaluated by comparison with freeze-drying processing.

The hydrodiffusion allowed to obtain a dried material with 12% moisture in 43 min when 550 g of broccoli by-products were used. Diffused water contained up to 317 µg/mL gallic acid equivalents of phenolic compounds, 11 mg/mL free sugars, 9 mg/mL amino acids, and 356 µg/mL glucosinolates, depending on the type of by-product used. MHG dehydration reduced the amount of carotenoids and chlorophylls in comparison with freeze-drying. Notwithstanding, this reduction was not observed for all other compounds evaluated, namely glucosinolates, phenolic compounds, protein, and total carbohydrates.

This work shows the potential of MHG for dehydration of broccoli by-products, allowing to obtain two ingredients: the water-soluble material recovered by diffusion and the dehydrated broccoli by-products, with valuable compounds for the formulation of functional foods. To obtain ingredients rich in carotenoids and chlorophylls freeze-drying can be hypothesized, but overall MHG technology has potential for industrial by-products stabilization and further valorisation.

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From maize flour to bread: changes in hydroxycinnamic acid and their derivatives after processing

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Maize (*Zea mays* L. ssp. *mays*) is one of the main cereals in the world[1]. Portuguese maize traditional varieties are used for the production of *broa*, a traditional Portuguese maize bread[2]. *Broa* is traditionally made with maize flour (50-100%) and rye and/or wheat flours (0-50%)[2].

Comparing to other cereals, maize contains a high level of phenolic compounds[1], which may be important in health[3]. However, these effects depend on their bioaccessibility that may regulate their bioavailability. Also the techniques used in maize processing may affect these effects[1]. In raw maize, less than 20% of the total phenolic content is present in the soluble form ("extractable polyphenols")[4]. These compounds have low-intermediate molecular mass and are more available for absorption in the human intestine than the insoluble compounds[5]. Thus, it is important to understand the changes that occur in maize phenolic composition after maize processing to maize bread.

In this study, 11 maize traditional open pollinated varieties and one commercial hybrid variety, as well as their corresponding maize breads and wheat and rye flours used in their preparation, were milled and flour extracts were prepared with ethanol:water (50:50 v/v), in order to analyse the soluble phenolics. Phenolic compounds were identified by liquid chromatography - tandem mass spectrometry (LC-MS/MS) and quantified by high performance liquid chromatography with diode array detector (HPLC-DAD). Free hydroxycinnamic acids, such as ferulic and *p*-coumaric acids, were identified in maize flours and breads, as well as in rye and wheat flours. Hydroxycinnamic acid amides, such as diferuloyl putrescine, were identified in both maize flours and breads. Maize-based breads showed a higher free hydroxycinnamic acids content than the corresponding flours (maize, wheat and rye), and a lower content in phenolic acid amides. Results are discussed considering the differences observed among the different varieties studied.

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Dragon fruits as an alternative source of colorants: evaluation of antibacterial activity and colouring capacity

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Betalains are a group of secondary metabolites named chromoalkaloids that are synthesized from tyrosine. These compounds have gained some attention in the last few years mainly due to their interesting bioactive potential, namely antioxidant, antimicrobial, and other bioactive properties [1]. Their strong and vibrant colours are also one of the characteristics by which these compounds have gained visibility in the food and pharmaceutical industries [2]. Betalains can be divided in two groups regarding the colour range: betaxanthins in the orange to yellow range, and betacyanins in the purple to pink range. Thereby, these compounds can be used as natural colouring agents, providing alternatives to the massively used artificial counterparts [3]. Although there are already some natural options in the market, these are not enough to meet the needs of the food industry, due to the growing concern of consumers regarding what they eat.

Thus, the objectives of this work were to: i) obtain bioactive extracts and with strong colouring capacity from the epicarp of two pitaya varieties, white-fleshed pitaya *Hylocereus undatus* (Haworth) Britton & Rose (WFP) and red-fleshed pitaya *Hylocereus costaricensis* (F.A.C.Weber) Britton & Rose (RFP); ii) chemically characterize the betalains' content through HPLC-DAD-ESI/MS; iii) evaluate the bioactive properties of the extracts, namely antimicrobial activity. The extracts were obtained through a dynamic maceration assisted by heat, and the betacyanins' profile was characterized by HPLC-DAD-ESI/MS. The antibacterial capacity was determined against a panel of Gram-negative and Gram-positive bacteria using the colorimetric method of rapid detection with *p*-iodonitrotetrazolium chloride (INT), and the responses obtained were expressed as minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations.

By the chromatographic analysis of the extracts it was possible to identify six compounds in both samples. For the WFP the major compounds identified were 6'-*O*-malonylbetanin (phylocactin), followed by 4'-malonyl-betanin, while for the RFP the major compound found was phylocactin.
In the evaluation of the antibacterial activity, both extracts showed MIC and MBC values that ranged from 10 to 20 mg/mL. Gram-positive bacteria showed to be more susceptible to both extracts then the negative strains.

Further studies need to be conducted to better understand the correlation between the bioactive potential and the betacyanins' composition. Nevertheless, these natural matrixes proved to be viable alternatives for obtaining colouring extracts with antimicrobial properties.

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Impact of Bioprocessing on Bioactive and Flavour Compounds of Berry Products

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Extensive research has demonstrated a wide range of health-promoting effects of berries, therefore consumption of berries has been highly recommended by professionals in the field nutrition and public health. However, berry consumption has been often limited on the one hand by the challenging sensory properties (taste and flavor) of fresh berries, and on the other hand by seasonal availability and short shelf life of fresh berries. Therefore processing of berries is crucial for improving sensory properties and shelf life of berry products in order to increase the consumption of berries.

The aim of the current study was to evaluate various biotechnological methods on their potential in processing of berries. Sea buckthorn (*Hippophaë rhamnoides* L.), blackcurrant, bilberry (*Vaccinium myrtillus* L.), and black chokeberry (*Aronia melanocarpa* L.) were chosen to be the target berries for investigation due to the widely shown health benefits and the special taste profiles characterized by high astringency and/or high acidity. Fermentation with various strains of Saccharomyces cerevisiae and non-Saccharomyces yeasts was performed to produce bilberry wines from bilberry juice. Malolactic fermentation was applied to decrease the content of malic acid in order to reduce the acidity and astringency. Enzymatic treatment is another important technology for bioprocessing of food. In this study, we investigated the enzymatic treatment in processing of blackcurrant juices as an example to demonstrate the impact of enzymatic processing on the composition and quality of berry juices.

Compared to *S. cerevisiae*, fermentation of bilberry juice with strains of *Torulaspora delbrueckii* resulted in lower levels of ethanol and acetic acid and higher content of succinic acid, lactic acid, and higher alcohols in the fermentation products. *Schizosaccharomyces pombe* strains produced more glycerol, acetaldehyde and/or pyruvic acid.[1] The choice of yeast strains play a significant role in determining the profile of volatile compounds in the fermentation products from bilberry juices.[2]

The strain *Lactobacillus plantarum* DSM 10492 converted all malic acid to lactic acid in sea buckthorn juice, and *L. plantarum* DSM 20174 in chokeberry juice. Fermentation with DSM 10492 reduced the content of flavonols by 9–14% and hydroxycinnamic acids by 20–24% in chokeberry juice without affecting the content of anthocyanins. Fermentation with DSM 10492 did not show any clear impact on the content of flavonol glycosides in the sea buckthorn juice.[3]

Enzymatic treatments increased the juice yield and the content of phenolic compounds in blackcurrant juices, but decreased the content of volatile compounds in blackcurrant juices, by 100-fold for terpenoids and 2-4 folds for esters.[4,5]

Our results showed that fermentation and enzyme-aided processing are potential bioprocessing methods for modifying the composition, and thus sensory properties of berry juices and beverages. The impact on composition and quality of fermentation products are highly dependent on berry species, strains of microbes, the enzymes as well as processing parameters.

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Flavonoid enrichment of fresh-cut apple through osmotic dehydration

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The demand for healthier food products has been increasing recently and the popularity of functional foods is gaining much interest among consumers. Flavonoids, such as quercetin and fisetin, are bioactive compounds that provide numerous health benefits, such as antioxidant and senolytic activity [1]. Low-calorie sugars may be used as an alternative to sucrose in order to reduce the risk of chronic diseases [2]. The objective of this study was to determine the feasibility of incorporating flavonoids and low-calorie sugar substitutes in fresh-cut apple via osmosis. Osmotic dehydration (OD) is a minimal processing technology that promotes mass transfer in fruits, while retaining its fresh-like sensory qualities [3].

In the present work, the osmosis-driven infusions of quercetin and fisetin into apple cubes were performed. The effects of different osmotic agents, sucrose and sorbitol and mannose, (mass ratio sample:osmotic solution of 1:4) on the OD mass transfer kinetics in apple cubes were studied at 25 °C and 40 °C for 8 hours. The colour and quercetin and fisetin contents were also analysed. Sorbitol and mannose were quantified during the OD process as well.

The results showed an increase in the kinetics of water loss (WL) and solute gain (SG) at 40 °C, as well as an increase in quercetin concentration at the end of the OD process with sucrose. However, this effect of the temperature was observed only for the SG in the OD with sorbitol-mannose. Moreover, the use of these solutes resulted in a higher WL, but a lower SG, in relation to sucrose. While samples treated at 40 °C tends to present a lower lightness (L* value) and a higher yellowness (b* value) than at 25 °C, there were no significant differences in the total colour differences (TCD) between both temperatures regardless the solute used. The use of sorbitol-mannose, on the other hand, contributed to lower decreases in lightness and higher increases in yellowness than sucrose, the latter most likely due to the solute gain, which included quercetin. There was a lower TCD in samples treated with sorbitol-mannose, which goes along with the results of the kinetics.

The results of the present work suggest that OD using alternative low-calorie and health promoting solutes is also an effective treatment to simultaneously enrich fresh-cut apple with senolytic flavonoids, resulting, therefore, in a great potential for a novel functional food.

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Effect of sweetener and storage on formation of compositional and sensory properties of jams

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The use of sugar substitutes is gaining an increasing popularity both in food industry and among consumers. Natural sweeteners or sweeteners originating from natural sources, such as different sugar alcohols, are in the centre of the interest. Our aim was to investigate the effect of different sugars and sugar alcohols on the sensory properties of blackberry and apricot jams during the storage period.

In this study apricot and blackberry jams were prepared using four different sweeteners: sucrose, fructose, xylitol and erythritol. Jams were stored at room temperature in a dark place for nine months. Measurements were performed in the 0, 1st, 3rd, 6th, 9th month after the production. Colorimetric measurements were accomplished in the CIE L*a*b* tristimuli coordinate system. The determination of sensory profile was achieved by a sensory panel of 12 trained members, according to standard requirements. Sensory attributes were also measured instrumentally, by electronic tongue and electronic nose. Antioxidant properties of the samples were determined over the storage period by different in vitro assays. Descriptive statistics, principal component analysis (PCA), discriminant analysis (LDA) and ANOVA test were used for the statistical evaluation of data in R-project and Microsoft Excel software.

Jams sweetened with erythritol significantly differed in taste and odour parameters from the others in case of classical sensory measurement, while jams sweetened with xylitol and fructose reached similar results compared to jams sweetened with sucrose. LDA results of electronic tongue showed separation tendency according to the storage time and clear separation according to the type of sweetener, especially in the case of blackberry jams. Antioxidant capacities did not change according to a clear trend during the investigation period, this was mainly attributed to formation of Maillard reaction products and polymeric compounds.

The type of sweetener has a measurable impact on the formation of both sensory attributes and antioxidant properties. Storage time also has a well-defined effect on the sensory and compositional properties of jams, this depending on the type of sweetener as well.

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Flavan-3-ols in apple pomace: role of their interactions with arabinans

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The occurrence of polyphenols in apple pomace, mainly flavan-3-ols, has been related to their capability to interact with polysaccharides, especially pectin [1, 2]. As pectin represents a collective of polysaccharides, including homogalacturonans and rhamnogalacturonans with galactan and arabinan side chains [3], the relative contribution of each structure in pectic/flavan-3-ols interactions deserves investigation. Given the simplicity of use, sugar beet arabinan (SB Ara) was selected as model to assess the ability of pectic arabinan side chains to interact with flavan-3-ols. To assess the effect of branching, sugar beet debranched arabinan (SB dAra) obtained by enzymatic treatment of SB Ara with arabinofuranosidase was also tested. Furthermore, an apple pomace arabinan (AP Ara) characterized by having ten-fold the amount of polyphenols detected in sugar beet arabinans was also evaluated to determined the effect of covalently bond polyphenols to the polysaccharide backbone on its interactions with polyphenols. Isolated flavan-3-ols from apple with an average degree of polymerization of 6 were selected for the interaction studies. The interactions were studied by Isothermal Titration Calorimetry (ITC) to assess, among others, enthalpy (ΔH), entropy (ΔS), and association constant (K_a). A phase diagram was established by measuring the turbidity of arabinan/flavan-3-ols solutions at different concentrations. The selectivity of these interactions towards the polyphenolic and/or polysaccharide structures was also evaluated by thioacidolysis and glycosidic linkage analysis of the supernatants and of the precipitate from ultracentrifugation of the ITC.

The interactions of flavan-3-ols with the arabinans presented ΔH and ΔS ranging from -2.1 to -24.6 $kJ.mol^{-1}$ and -33 to 45.4 $J.mol^{-1}.K^{-1}$. This demonstrated that the interactions were exothermic, mostly driven by hydrophobic interactions, in addition to hydrogen bonding. For SB Ara and SB dAra the association constants (K_a) were 540 and 391 M^{-1}, suggesting that less branched arabinans have higher affinity towards flavan-3-ols. For AP Ara the lowest K_a was observed (85 M^{-1}), suggesting the covalently bond polyphenols impair further interactions of the polysaccharide backbone with polyphenols. These observations agreed with the higher turbidity values observed for SB dAra than for other arabinans when incubated with flavan-3-ols. Insoluble arabinan/flavan-3-ols complexes were formed with flavan-3-ols of higher DP and with the less branched polysaccharides.

These results demonstrate the contribution of arabinans for flavan-3-ol retention in apple pomace limiting their transfer for the juice.

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Quality of protein concentrates isolated by two different methods from Baltic herring (*Clupea harengus membras*) and roach (*Rutilus rutilus*)

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Baltic herring (*Clupea harengus membras*), a subspecies of the Atlantic herring, is commonly found in the Baltic Sea region. In Finland, Baltic herring is commercially the most important fish species with annual catch exceeding 100 million kilograms, but only a fraction of this catch is used as food. Roach (*Rutilus rutilus*) is a cyprinid fish and considered to be a low-value species. Roach is therefore currently highly under-utilized. [1]

One major factor limiting the use of these fish species as food is that they are difficult to process due to the small size of Baltic herring and abundance of small bones in roach. One possibility to improve the utilization of these fishes is to extract their proteins and incorporate the protein fraction into food products. The pH-shift process, based on solubilization of protein at a high or low pH and precipitation at its isoelectric point, has been successfully used to isolate proteins from several fish species, including Baltic herring [2]. Enzymatic extraction is based on hydrolysing the protein with proteases, therefore making it more water-soluble, and has also been employed for extraction of fish protein [3].

The aim of the study was to compare the quality of proteins and lipids in protein concentrates from Baltic herring and roach, produced by two different fractionation methods. Protein was extracted by alkaline and acidic pH-shift processes and enzymatic hydrolysis using three different endoproteases. The nutritional quality of the protein concentrates was analyzed focusing on protein and lipid content, and amino acid and fatty acid composition. Peroxide value, protein carbonyls and volatile compounds were measured as indicators of oxidation.

Results of the study suggest that the two extraction processes, as expected, produce significantly different protein concentrates. Protein content of the concentrates ranged from 67% to 87% protein (on dry weight basis), the enzymatically fractionated samples having slightly higher protein contents than the pH-shift samples. The protein concentrates produced by pH-shift processes from both fishes contained a significantly higher amount of lipids (11% and 19% for roach and 17% and 17% for Baltic herring protein concentrates from acidic and alkaline processes, respectively) compared to enzymatically fractionated concentrates (5.5 - 6.0% and 3.2 - 3.5% for roach and Baltic herring protein concentrates, respectively). Differences in amino acid and fatty acid compositions suggest that enzymatic fractionation and pH-shift process extracted different proteins and lipids from the fishes. Protein concentrates from pH-shift processes tended to have a higher essential:non-essential amino acid ratio. The protein concentrates produced by pH-shift processes showed more signs of oxidation compared to enzymatically fractionated ones.

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Interaction of human salivary proteins with food polyphenols

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Polyphenols have been associated to health benefits (e.g. cardiovascular protection, anticancer activities) of plant-derived food and beverages (e.g red wine and red fruits). Some polyphenols, tannins, are known for their ability to interact with (biological) proteins. The oral cavity is the first contact between human body and polyphenols. One of the first interactions' of polyphenols inside oral cavity is toward salivary proteins (SP). This interaction is important both at sensory (astringency sensation) and at biological (polyphenols bioavailability and/or inhibition of digestive enzymes) levels[1, 2]. Furthermore, several factors influence this interaction, e.g.proteins/polyphenols structure, pH and ionic strength. Among the several families of SP, basic proline-rich proteins (PRPs) class has been identified as the major one with high interaction with polyphenols[3]. There is not much information about the interaction of other classes of PRPs or even with other families of SP.

So, in this work, it was intended to study the interaction between different families of SP (acidic PRPs, basic PRPs, glycosylated PRPs, statherins, cystatins, P-B peptide) and polyphenols from different classes and with different structural features (procyanidin dimer B2, procyanidin dimer B6, procyanidin dimer B2-gallate, punicalagin, castalagin, vescalagin and malvidin-3-glucoside). These interactions were studied by Saturation Transference Difference-Nuclear Magnetic Resonance (STD-NMR), isothermal microcalorimetry (ITC) and computational studies[4, 5]. The combination of these techniques allowed the characterization of the interactions from low to high polyphenol/SP ratios. This study allowed to characterize these molecular interactions by determination of association constants, thermodynamic parameters, binding stoichiometry and structural epitopes involved in the binding. Both techniques are in agreement that P-B peptide was in general one of the SP with higher affinity for all polyphenols.

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Extraction of phenolic compounds by high hydrostatic pressure from eight edible algae species from the North-West coast of Spain: Process modelling and optimization

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Two thirds of the word are covered by oceans and a vast majority of that upper layer are inhabited by photoautotrophic organisms such as algae. Algae are not only of high ecological, but also of great economic importance. The industrial exploitation of algae has suffered a boom in the last two decades, revealing a source of compounds relevant to a diverse range of sectors (agriculture, energy, food science, cosmeceutical, pharmacology, etc). In consequence, many possible applications have emerged, such as production of biofuels for energy production, as nutraceutical ingredients in fish meals, as fertilizer in landfill applications, anti-biofilm activity in food science, incorporation in cosmeceutical and pharmacological products due to the rich content in bioactive compounds, among others [1].
In this work, high hydrostatic pressure (HHP) was applied to the extraction of phenolic compounds from eight edible algae species (Table 1).

Table 1. Edible algae species used for this study.

Local common name	Scientific name
Kombu	*Laminaria spp.*
Kombu real	*Saccharina latissima*
Espagueti de mar	*Himanthalia elongata*
Wakame	*Undaria pinnatifida*
Nori	*Porphyra spp.*
Dulse	*Palmaria palmata*
Alga percebe	*Codium spp.*
Lechuga de mar	*Ulva spp.*

The process was optimized by response surface methodology using a five-level central composite design combining the independent variables of processing time (t, 5-90 min), pressure (P, 10-600 MPa) and solvent (S, 0-100 % of ethanol, v/v) [2]. The individual and grouped phenolic compounds were analysed, and the extraction yield were used as response variables. The theoretical models were fitted to the experimental data, statistically validated, and used in the prediction and optimization steps. In general, the optimum extraction conditions for phenolic acids for all eight species analysed were found at shorter values of t, high values of P and high values of S. The identified phenolic compounds were also clustered according to the conditions that maximize their extraction. HHP was highlighted as a promising emerging technology to extract phenolic compounds from edible algae species using a green solvent and reduced extraction times.
The analysis presented provides important data that allows the comparison between different extraction conditions, in terms of efficiency, and consequent related decision making. In an industrial level, these methodologies reduce costs related to energy, solvent consumption, equipment investment, etc. Achieving the optimal conditions and maximizing the responses is an important step to guide the choice of a suitable and sustainable process. In conclusion, the present study contributes in the valorisation of edible algae species, common in the North-West region of Spain, by the obtainment of rich extracts in phenolic compounds that potentially can be applied as ingredients in different industrial fields.

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Pomegranate peel extraction optimization and characterization and their inclusion in carrot juice improving their safety and quality

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Annually one-third of the food produced worldwide for human consumption, corresponding to approximately 1.3 billion tonnes, are wasted representing a cost of around 990 billion dollars [1]. Thus, there has been a social, political and environmental pressure to improve profitability and valorization of fruit by-products [2] since they are very rich in high-value compounds, such as bioactive compounds that can be used by the food industry as flavourings, colourants, and texturizer additives as well as antioxidants or antimicrobials agents [3].

This work aimed to valorise the pomegranate peel through the incorporation of its extracts in carrot juice. High pressure (HP) assisted extraction was performed to obtain a pomegranate peel extract that was analysed regarding their antioxidant activity, total and individual bioactive compounds and their antimicrobial activity. Thereafter, the extract (5 mg extract/mL juice) was added to raw carrot juice, which was subsequently pasteurized by HP processing and conventional thermal processing and stored at 4 °C for 28 days, where they were monitored for several quality and safety parameters.

Optimum HP extraction conditions allowed to increase extraction yields of several compounds (Table 1) and extracts presented antimicrobial activity against 7 pathogens but not inhibited the microbial growth of 5 lactic bacteria. Fortified juices showed lower counts ($p<0.05$) than the non-fortified ones for total aerobic mesophiles (Figure 1) and psychrophiles. No yeasts and moulds were detected during storage. The extract supplementation increased total soluble solids, lowered pH values of carrot juice ($p<0.05$) but in general did not affect ($p>0.05$) any colour parameter (L*, a*, b*, and ΔE*). Total phenolics, flavonoids, and hydrolysable tannins contents, as well as antioxidant activity were always higher ($p<0.05$) in supplemented juices during the 28 days of storage.

Table 1. Optimized extraction conditions for each parameter.

Variables	Total yield	ABTS	DPPH	FRAP	Total phenolic	Total anthocyanins	Total flavonoids	Total tannins
Pressure (MPa)	382	406	525	356	470	395	492	600
Extraction time (min)	30	30	30	30	30	23	30	30
Ethanol concentration (%)	36	41	40	32	55	80	37	36
Increase (%)	6	12	13	7	13	5	18	6

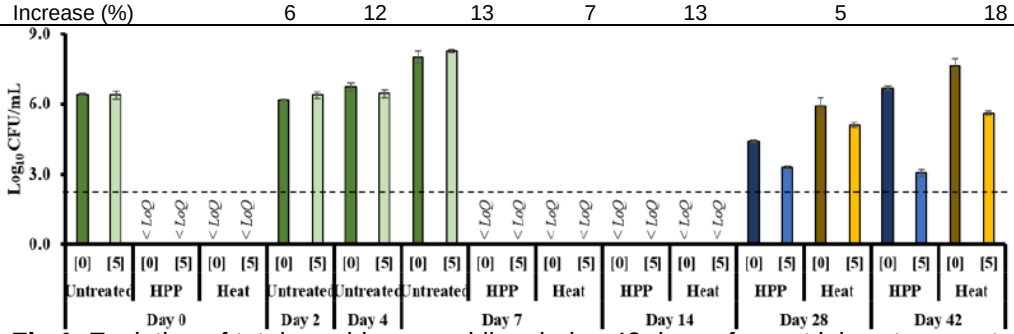

Fig.1. Evolution of total aerobic mesophiles during 42 days of carrot juice storage at 4°C.

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ORAL COMMUNICATIONS

Food Safety

The antioxidant efficiency in O/W emulsions can be controlled by modulating antioxidant interfacial concentrations

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Control of lipid oxidation is important in the development of specific strategies for preparing healthier and more nutritional foods with longer shelf lives [1]. Addition of antioxidants (AOs) to food products is one of practical strategies used in the food industry to retard or inhibit lipid oxidation. The antioxidant efficiency depends on both the rate of the reaction with the radicals (whose value depends on the chemical structure of the AO and the medium properties) and on their local concentrations at the reaction site, which in emulsions is believed to be the interfacial region but not proved experimentally so far.

Here we have employed a homologous series of antioxidants derived of gallic acid to analyze the effects of AO hydrophobicity on the oxidative stability of stripped fish oil-in-water emulsions and on their distributions in the intact emulsions [2]. The effects of parameters that may affect the distribution of the antioxidants and, eventually, their efficiencies have also evaluated such as the effects of the hdyrophobicity of the AO, emulsifier concentration (Φ_I) and the oil-to-water (O/W) ratio employed in the preparation of the oil-in water (O/W) emulsions.

Results show that the efficiency of gallates increases upon increasing their hydrophobicity up to a maximum (3-fold) at the octyl derivative, after which the efficiency decreases. The observed parabola-like variation in the efficiency with the AO alkyl chain length parallels that of the percentages of AOs in the interfacial region but does not parallel that in the oil region. Interfacial concentrations are 20-100 times (depending on Φ_I) higher than the stoichiometric (added) antioxidant concentration, oil concentrations are similar or slightly higher (1-6 fold) meanwhile aqueous concentrations are smaller (0.8-10 fold), highlinging that the compartmentalization effects play a key role in defining the AO response against the oxidation of lipids. The effects of O/W ratio on the interfacial concentrations are complex and depend on the AO hydrophobicity. Changes in the O/W ratio can also be used to optimize the interfacial concentrations of hydrophobic (octyl and lauryl gallates) or hydrophilic (gallic acid) AOs, but not those of AOs of intermediate hydrophobicity (propyl gallate). Results provide, for the first time, experimental evidence that the variations in the efficiency of homologous series of AOs in emulsions are due to differences in their interfacial concentrations, confirming that – other things being equal- there is a direct relationship between the AO concentration in the interfacial region of emuslions and its efficiency [3]. A careful choice of the parameters controlling the distribution of AOs is crucial because they strongly affect the availability of AOs at the reaction site.

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Mycotoxin content of *Salicornia* L. in Portugal

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Salicornia L. is a genus of annual, fast growing, halophytic plants, with reduced leaves and succulent, fleshy, articulated stems. S*alicornia* spp. are some of the most salt-tolerant land plants, and can be found in both coastal salt marshes and inland saline habitats, in almost any part of the world. They have been used since ancient times in Korean, Japanese and Chinese diets, as a seasoned vegetable, and also as a traditional medicine used to treat a variety of diseases, such as cancer, diabetes and obesity. In Europe, its consumption is not yet widespread, but its popularity has been growing, especially in gourmet cuisine, as well as in traditional gastronomy as an alternative to salt. Indeed, the nutritional value and mineral richness of *Salicornia* make it a very promising functional ingredient. In addition, *Salicornia* is already regarded as the "plant of the future" owing to its great potential as a crop in deserts and saline soils [1]. However, amidst this positive scenario of valorisation of this resource, other aspects, such as the presence of food safety hazards, need to be considered.

Mycotoxins are toxic metabolites produced by certain fungal species that can grow on a variety of plant crops and are associated with morbidity and mortality in humans and animals [2]. Mycotoxin production in *Salicornia* can occur at any stage of the supply chain. Poor agricultural and harvesting practices, as well as inappropriate drying, milling, packaging, transport and storage conditions promote fungal growth, thus raising the risk of mycotoxin contamination. Furthermore, the salt marshes environment, being suitable for *Salicornia* development, also provides favourable conditions for the growth of toxigenic fungi and their mycotoxins production [3]. Notwithstanding this, to the best of our knowledge, no study has been published so far, to assess the extent of the problem of mycotoxin contamination in *Salicornia* spp.

Thus, the aim of this study was to verify the presence of potentially mycotoxigenic fungi, and to investigate the presence of aflatoxins and ochratoxin A in wild and commercially available *Salicornia* in Portugal. Wild samples were collected in two different areas of the Mondego estuary (south arm and Morraceira island) in two different time periods (July and November). Both fresh and powdered commercial samples were analysed. The mycotoxin contamination levels were determined with UPLC-TOF-MS.

The results showed important levels of contamination of *Salicornia* by aflatoxins, namely, in commercial samples, which could pose a threat to public health. Current European legislation does not cover *Salicornia*, and, in view of the results obtained and of its increasing consumption, it is imperative to establish maximum levels of contamination, to carry out an effective monitoring and to adopt measures that warrant risk minimisation. These may include the reduction of ground contact or an appropriate control of the transport and storage conditions, or even the choice of harvest time. Whatever the case may be, it is important that further studies be conducted, to evaluate fungal and mycotoxin contamination along the entire production and distribution chain.

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Development of an analytical method for the simultaneous measurement of 10 biogenic amines in meat. Application to Beninese grilled pork samples.

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The formation of biogenic amines is essentially the result of enzymatic decarboxylation of specific amino acids, due to microbial enzymes. Knowing that biogenic amines, particularly histamine and tyramine, can cause health problems, their presence in food is not desired. What is more, quantification of biogenic amines in meat is suitable for detecting the stage of deterioration of meat and their concentration may be associated with the freshness and the level of degradation of proteins of the product. Therefore, a UPLC-Fluorescence method has been developed to evaluate the concentration of ten biogenic amines as dansylated derivatives. Two Reference Materials incurred with histamine were used to evaluate the performances of the method. The averages of the measured values were evaluated at 98.7% and 96.8% of the expected values, for the two materials.

The developed method was applied to quantify biogenic amines in grilled meat from Beninese markets. The biogenic amines index was calculated for each sample. In this study, ten samples can be considered as fresh with values lower than 5 mg/kg, while one sample is considered as acceptable (16.9 mg/kg) and one sample is considered as spoiled (82.8 mg/kg). No link between the biogenic amines concentrations and the cooking conditions was observed. Because the biogenic amines are not heat sensitive, the measured concentrations of biogenic amines in this study could be explained by bad hygienic conditions during meat storage before cooking. It means that the Beninese population may be exposed to sometimes high biogenic amines content, leading to allergies or other more serious health problems.

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Health promoting foods with sea buckthorn: more benefits, less acrylamide

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Puffed breads from different cereals and pseudocereals (wheat, spelt, corn, rye, oat, amaranth, buckwheat, rice) possess of many nutritional advantages, especially in case of wholemeal base. Moreover, addition of sea buckthorn *(Hippophae rhamnoides L.)* powder rich in many bioactive compounds significantly enhances functional properties of these products in such parameters as antioxidative properties, rutin and quercetin levels, essential amino acid content and unsaturated fatty acid composition. On the other hand, high level of the L-asparagine amino acid in cereal bran in the case of wholemeal base raw material as well as in sea buckthorn powder predetermines considerable acrylamide formation during the extrusion process conducted at temperatures between 220 and 260°C.

Since the acrylamide is considered to be a probably carcinogenic compound, Commission Regulation No 2017/2158 recently established mitigation measures and benchmark levels for acrylamide reduction in food. Acrylamide is also regulated in puffed products with a benchmark level set on the value of 300 µg/kg. For this reason, all potentially risky products should be evaluated in terms of acrylamide presence and appropriate tools should be applied to minimize this risk. Our experience with the application of mitigation measures in industrial production of innovative extruded breads will be presented in this contribution.

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Is it safe to eat seafood? A case study of flame-retardants

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Brominated flame retardants (BFRs) are organobromine chemicals with an inhibitory effect on combustion and tend to reduce the flammability of industrial and household products. Among several toxicological effects, many of these substances are recognised endocrine disruptors [1]. Polybrominated diphenyl ethers (PBDEs) comprise one of the most relevant classes of BFRs and they can find their way into the environment by different routes and processes. The dietary route, through seafood consumption, is a main contributor to human exposure [2]. Therefore, several surveys have been undertaken to estimate the severity of seafood contamination with PBDEs, but also with their biologically active metabolites – methoxylated PBDEs (MeO-PBDEs) [3,4]. In addition, assessing the impact of cooking as a mitigation strategy as well as studying *in vitro* bioaccessibility of these toxicants became a subject of urgent matter.

Hence, the aim of this work was to provide thorough information on the dietary pathway of PBDEs and MeO-PBDEs after consumption of contaminated cooked seafood.

For the purpose, environmental-friendly, cost-effective and fast methods were developed and validated for the analysis of PBDEs and MeO-PBDEs using a Quick, Easy, Cheap, Efficient, Rugged and Safe (QuEChERS) extraction approach for solid samples and a Dispersive Liquid-Liquid Microextraction method (DLLME) for plasma. Instrumental analyses were performed with GC-MS/MS [5].

Afterwards, selected seafood (with diverse lipid content) were cooked using normal household practices (steamed, grilled, and microwaved), which resulted in considerable loss of targeted pollutants (average 20 % loss). Finally, an *in vitro* method that simulates four human adult digestion steps with distinct digestion times, pH, and composition (simulating saliva, stomach digestion, small and large intestine digestion) was applied to raw and cooked fish. Bioaccessibility of PBDEs and MeO-PBDEs was low (below 20%), as predicted, which point to a likely heavy impact on gut microbiota.

The results achieved herein are of great value for the prediction of both amounts and nature of the BFRs that the seafood consumers may be exposed after the ingestion of contaminated food ingestion as to ascertain the true impact on human health.

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Selecting alternatives to chlorine for strawberry sanitation while maintaining nutritional and physicochemical quality

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Strawberry production in Europe exceeds 740,000 tones and it is a highly consumed product, both fresh and frozen [1]. Fresh strawberries have a short life, and losses due to microbial spoilage can range up to 53% [2]. The increasing evidence suggesting that phytochemicals present in strawberries have health-protecting effects make this fruit attractive for consumers [3]. However, in order to minimize the risk posed by outbreaks associated with berries [4] and to avoid the safety issues related with by-products from chlorine disinfection in fruit industry, this work aims to search for alternatives to disinfect strawberries while maintaining their quality and nutritional parameters.

Water-assisted ultraviolet C light (WUVC) was tested to reduce natural contamination and pathogens on strawberries. For this, a prototype consisting in 4 UV lamps immersed in a water tank with an agitation and aeration system was used. Four washing conditions were tested, combining the use of 2 or 4 lamps for 1 or 5 min. Tap water and NaOCl 200 ppm were used as negative and positive controls, respectively. In this case, yeast and molds (Y&M) were not affected by UVC light, and 1 min-treatment was ineffective to significantly reduce total aerobic mesophylls (TAM). Nonetheless, 3 log reductions of *Listeria innocua* were achieved after WUVC treatments, comparable to those resulting from chlorine-wash. Moreover, UVC light was useful to allow water recirculation if needed, as microorganisms remaining in washing water were minimized after irradiation.

Peracetic acid (PA) was also proposed as a sanitizing agent in this work. Strawberries, were washed for 1 or 2 min in solutions containing 20, 40 or 80 ppm PA. Tap water and NaOCl 200 ppm were used as treatment controls. Results showed that washing time was irrelevant to reduce artificially inoculated *L. innocua,* TAM, and Y&M*. L. innocua* population was reduced at least 4 log units by all washing treatments, and remaining counts in PA washing solution were 4 log units lower than they were in control water.

In both cases, quality parameters including pH, total titratable acidity, texture and color, biochemical analysis including antioxidant activity and phenolic compounds, and nutritional aspects such as vitamin C or organic acids, were not significantly affected by sanitizing treatments, thus maintaining quality and nutritional values of disinfected strawberries.

With these results, a combination of both strategies was carried out simultaneity to improve effectiveness of strawberry disinfection and to reduce sanitation time needed with WUVC equipment. Hence, WUVC irradiation using 4 lamps in combination with PA at 40 or 80 ppm and applied for 2 min resulted better than individual treatments in terms of microbial reduction. Future perspectives include to study the suitability of this hurdle approach to sanitize strawberries for different purposes, such as ready-to-eat fruit or frozen products.

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Investigation of diet-related DNA adduct formation by means of DNA adductomics

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Epidemiological research has demonstrated that red meat consumption contributes to colorectal cancer (CRC) risk. Different hypotheses have been put forward to explain this causal relationship, but the heme hypothesis, stating that heme iron in red meat stimulates the formation of genotoxic N-nitroso compounds (NOCs) and lipid peroxidation products (LPOs), has been receiving the most support. Both NOCs and LPOs can exert DNA damaging effects like e.g. DNA adduct formation, which is in fact believed to be the first step in chemically induced carcinogenesis [1,2].

We developed a novel DNA adductomics method to enable in-depth investigation of diet-, and specifically, NOC- and LPO-related DNA adduct formation. To this purpose, an in-house diet-related DNA adduct database and a high resolution mass spectrometry (HRMS) based methodology were established [3]. After successful validation, the state-of-the-art DNA adductomics platform was implemented to investigate the genotoxic effects of red vs. white meat digestion. Beef (model for red meat) and chicken (model for white meat) were digested *in vitro* (static model) as well as *in vivo* (Sprague-Dawley rats), after which the DNA adductome of digestion samples (*in vitro* setups) and tissues (*in vivo* setup) could be mapped. Data processing and statistical interpretation was performed with Xcalibur™, ToxFinder™, SPSS, Sieve™, and Simca™.

Combining the results from 3 independent *in vitro* and 1 *in vivo* digestion experiment(s), 7 DNA adduct types, including O^6-carboxymethylguanine, dimethyl- or ethylthymine, methylguanine, heptanalguanine, a malondialdehyde-guanine adduct, and a malondialdehyde-cytosine adduct could be singled out as potential red meat digestion markers [4-7]. The discovery of these red meat digestion related DNA adduct markers is highly relevant to the red meat-CRC hypothesis because their formation may be linked to DNA alkylation and/or oxidation by e.g. NOCs and/or LPOs. Follow-up research is to further investigate the role of DNA adduct formation in the red meat-CRC pathway as well as the mutagenic potential and human *in vivo* relevance of the proposed DNA adduct markers.

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Liquid chromatography/mass spectrometry and dielectric barrier discharge ionization (DBDI): a versatile tool for pesticide analysis in food

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Dielectric barrier discharge ionization (DBDI) has gained attraction in recent years as a versatile ionization method available in different formats) (ambient ionization probes, GC-MS, LC-MS or CE-MS interfaces, intended for many applications including ambient mass spectrometry imaging, environmental analysis, biological and pharmaceutical and food safety. The (dielectric barrier) discharge is typically formed between two electrodes, with at least one dielectric layer which separates the electrode from the plasma.

DBDI is an efficient ionization technique for LC-MS coupling as was first reported by Franzke and co-workers [1]. The DBDI probe can be easily implemented into commercial API sources so that the LC eluent was nebulized and vaporized in the same manner as for API. These features lead to enhanced analyte coverage compared to commercially available LC-MS sources.

Depending on the particular chemical class, the determination of some of the pesticides might be carried out by either GC-MS or by LC-MS. Among the large amount of pesticide classes, some of them are thermolabile (so they might be degraded in the GC injection portal) but they do not have the right moieties to be efficiently ionized by electrospray. As alternative to current commercial sources, new ionization sources based on dielectric barrier discharge ionization (DBDI) have been reported. DBDI sources feature different ionization mechanisms that include electron capture and proton transfer [2].

In this presentation, data from a thorough evaluation of DBDI as ionization interface for LC-MS for pesticide analysis in food is shown, which reveals attractive advantages over ESI and APCI provided its singular ionization mechanism versatility. Over 100 pesticides across a wide range of physicochemical properties were selected and the results were compared with both electrospray and atmospheric pressure chemical ionization (APCI) sources. Matrix effects, analyte coverage, sensitivity and salt adduct formation were evaluated with the three different approaches. The ability of DBDI to efficiently ionize challenging compounds such as captan, o-phenylphenol, bromophos ethyl, dazomet, and dinocap is also demonstrated.

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Glycoalkaloid profiles of herbal infusion using neutral loss - high resolution mass spectrometry

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Herbal infusions are consumed worldwide thanks to their "natural" beneficial effects; however, they often need specific controls to evaluate the possible presence of alkaloids (alks) [1]. Alks, widespread in nature, are nitrogen-containing organic constituents occur mainly in plants as secondary metabolites. These compounds can arise from amino acid metabolism or from amination of other substrates, which may be, for example, terpenes or steroids [2]. The biosynthesis often involves a further glycosylation of the alks (aglycones) as glycoalkaloids (glyalks) [3]. Alks have long been studied due to their specific toxicological characteristics, as some of them are suspected of having very dangerous properties, although the corresponding glycosylated forms are only rarely considered.

This study aims to investigate the profile of glycoalks belonging to the most important chemical classes (indole, piperidine, protoalkaloid, pyridine, pyrrolidine, pyrrolizidine, quinoline, steroidal, terpenoid, and tropane alks) of a wide selection of commercial herbal tea products (n=120), using a UHPLC equipped with an SPE on-line system and performing the separation on a byphenyl column. The binary mobile phase was composed of 0.1% formic acid (FA) with 5mM ammonium acetate (AAc) and MeOH/ACN 95:5 v/v with 0.1% FA and 5 mM AAc. A Full MS/AIF/NL dd-MS^2 experiment was performed in positive ion mode with the resolution set at 140,000 FWHM (m/z 200; 1.5 Hz) for full MS spectra, at 70,000 FWHM (3 Hz) for AIF and at 17,500 FWHM (12 Hz) for dd-MS^2 [4]. Neutral losses of pentose (m/z 132.0423), deoxyhexose (146.0579), hexose (162.0528), and of all the combinations of up to four of these sugar units were considered.

26 glycoalks were detected, including 9 new glycoalks never before reported in the literature. 28% of samples presented at least one glycalk, being *Escholtzia californiea* the richest one.
This study indicated the presence of the glycosidic forms in several commercial herbal teas used for domestic infusion, permitting higher awareness of the possible risks and benefits relating to the consumption of these products.

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ALGAL CONSUMPTION: WEIGHING BENEFITS AGAINST RISKS

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Macroalgae or seaweed are a fundamental ocean resource, which mankind has always known and used. Seaweeds are currently used for various purposes: as food —especially in *sushi* and other traditional recipes—; as biofertilizer; as a source of colloids; or for extraction of bioactives with antimicrobial, anticancer, and other biological activities. However, there are still many undervalued and insufficiently characterized seaweed species. This comprises edible brown seaweed (e.g. *Halopteris scoparia*, *Petalonia binghamiae*, *Saccharina latissima*), green seaweed (e.g. *Chaetomorpha linum*, *Ulva* spp.), and red seaweed (e.g. *Osmundea pinnatifida*) species. Recent studies [1-3] have shown that some of these seaweeds are rich in antioxidants, minerals, iodine, and display meaningful anti-inflammatory activities. On the other hand, there are also some public concerns, such as arsenic content. All these aspects have a variable and, sometimes, opposite impact on health, which needs to be adequately assessed. Thus, it is important to weigh benefits *versus* risks in algal consumption. Hence, this study sought to assess the main risks (As, excessive I) and benefits (adequate I, EPA+DHA) associated to the consumption of a representative array of less known seaweed species with a potential for the food/nutraceutical industry.

The methodology used in this evaluation was the most rigorous, based on the most advanced mathematical-statistical modelling [4] and the available data of As, I, and EPA+DHA contents in seaweeds [1-3]. This enabled the evaluation of the risk-benefit binomial for different consumption scenarios using thresholds such as EPA+DHA recommended daily intake (RDI), I RDI, or I Tolerable Upper Intake Level (TUIL).

This work led to advances in the assessment of possible consumption levels of the selected seaweed species. Namely, concerning I, species such as *Halopteris scoparia* and *Petalonia binghamiae* provide the full benefit with less than 20 g dry seaweed/week or 100 g steamed seaweed/week. Regarding As, there must be some caution, taking into account the geographical source of the seaweeds. Nonetheless, for seaweeds of less polluted areas, benefits seem to overweigh any possible risk.

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Multidisciplinary characterization of selected wheat genotypes and in-silico risk assessment of their potential toxicity for celiac disease patients

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Over the last years, important efforts have been devoted to develop technological approaches for wheat detoxification, the sourdough fermentation being the most promising route, also including a biotechnology strategy for the complete gluten degradation prior to consumption [1]. As main drawback the enzymatic detoxification approach causes the detrimental alteration of the technological properties as well. The identification of wheat genotypes with reduced gluten content and having naturally low amounts of epitopes toxic for celiac patients was recently re-evaluated as option for breeding practices and for the development of new detoxification strategies. Indeed, the varietal selection undertaken by breeders in the last decades caused a considerable impoverishment of the genetic diversity of wheat varieties present on the market. Starting from this, the researchers are encouraged to investigate the natural diversity of available wheat genotypes in light of their potential to encode a lower number of celiac disease epitopes [2].
In this contribution, we present the detailed characterization of a tetraploid wheat collection by an integrated analytical approach, scouting for candidate genotypes, which combine the potential lower toxicity/immunogenicity with conserved yield and rheological properties to encompass the perspective usability for bread or pasta making [3]. A preliminary profiling of gluten proteins was accomplished by R5-ELISA and HPLC-UV, focusing on the gliadin fraction as main responsible for immunoreactivity in celiac disease patients. In addition, data on grain protein content, grain yield per spike, dry gluten and gluten index were collected in order to provide complementary information about productivity-related traits and quali-quantitative characteristics related to wheat nutritional value and its technological properties [3]. The statistical evaluation of the whole data pool drove the selection of a short list of candidate genotypes that were subjected to a systematic proteomic characterization. Firstly, both the water-soluble and water-insoluble protein fractions of each genotype was characterized by means of typical bottom-up approach with the aid of either one or two specific cleavage enzymes (chymotrypsin and thermolysin) and software based HR-MS/MS spectra identification. In addition, the raw flour of each genotype was subjected to *in-vitro* simulated human gastroduodenal (GD) digestion according to the standardized static protocol proposed by Minekus et al. in 2014 [4], aiming at identifying peculiar amino acid sequences of GD resistant peptides. Finally, an in-silico risk assessment of potential toxicity for celiac disease patients was performed according to the most recent guidance provided by EFSA [5].

The integrated approach confirmed that durum wheat breeding programs accomplished in the last decades improved the gluten strength, without causing an increment of toxic epitopes. Even if none of the selected genotypes can be considered safe for CD patients, a lower toxicity level could be envisaged and, in perspective, they may represent an innovative solution in genetically predisposed individuals who may develop CD after prolonged gluten consumption.

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Comparative study of multiplex real-time Recombinase Polymerase Amplification and ISO 11290-1 methods for the detection of *Listeria monocytogenes* in dairy products

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With 320,000 human cases reported every year, foodborne zoonotic diseases continue to be a global public health threat. Dairy products, particularly those prepared from raw milk, have been implicated in foodborne infections due to different pathogens [1]. Among them *Listeria monocytogenes* is of particular concern due to its ubiquity, resistance to sanitation processes and high mortality rate [2]. These premises potentiate the development of novel methods which allow rapid detection of this bacterium.

Molecular techniques based in DNA detection, such PCR, have emerged to substitute the traditional culture based analysis employed nowadays, to reduce the time of analysis, which takes more than 4 days [3]. Isothermal amplification techniques, among them Recombinase Polymerase Amplification (RPA) have attracted interest by avoiding the use of complex equipment, allowing higher speed, reduced costs and its easier implementation in lab-on-chip systems [4]. The inclusion of an Internal Amplification Control (IAC) in DNA amplification methods has been widely recommended to increase the reliability of the results [5], but up to now no single study has approached the development of a multiplex real-time RPA method targeting *L. monocytogenes* with an IAC in food/ dairy samples.

In this presentation, the development and evaluation of a novel multiplex real-time RPA method, which includes an internal amplification control is reported, for the detection of *L. monocytogenes* in milk and different milk-based products. The methodology was compared against the reference ISO 11290-1 method, in order to better asses its performance in dairy products and implementation in food industry.

A limit of detection below 10 cfu/ 25 g (mL) was reached, and values higher than 90 % were also obtained when evaluated the relative sensitivity, specificity, accuracy, positive and negative predictive values and the index kappa of concordance. The molecular method allowed for a significant time reduction when compared with the ISO one, in addition of being a compatible approach as the same enrichment broth, half Fraser, was selected. Thus all positive samples were susceptible of being confirmed by the standard protocol, as recommended by the ISO 16140 for the validation of alternative methods.

In conclusion, the multiplex real-time RPA method, for the specific detection of *L. monocytogenes*, and an IAC, was successfully developed and evaluated and the results obtained by the developed methodology were comparable to those of the reference method ISO 11290-1, being a faster approach.

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Potential of thermosonication as an alternative to chlorine disinfection in strawberries inoculated with *Listeria innocua*

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BACKGROUND: Although strawberries are generally considered as safe, the concern about microbiological safety of fresh, minimally processed, and frozen strawberries has increased in recent years due to the emergence of several outbreaks of foodborne pathogens linked to their consumption [1]. Chlorine, which has traditionally been used for the disinfection of fruit and vegetables, has now been banned in several European countries because of health issues. This has led to increased interest in innovative physical and chemical disinfection strategies that include sonication and thermosonication, which is a combination of ultrasound processing and mild temperatures.

OBJECTIVES: The aim of the current study was to assess the potential of thermosonication as an alternative to chlorine disinfection of strawberries. Studied parameters included temperature (20-55 ºC), ultrasonic frequency (35-130 kHz), and processing duration (1-15 min) at an effective ultrasonic power of 250 W.

METHODS: Antimicrobial effects of thermosonication were assessed *in vitro* and *in vivo* using strawberries artificially inoculated with *Listeria innocua* CECT-910. The effect of thermosonication on the strawberries native microbiota and on quality parameters including colour, texture, visual appearance, phenolic content, and antioxidant capacity were also studied following previously described methods [2, 3].

RESULTS & DISCUSSION: *In vitro* trials demonstrated that both temperature and processing time significantly influenced the inactivation of *L. innocua* in water (p<0.001). When assessed *in vitro*, ultrasounds had no impact on the lethal effects of temperature on *L. innocua* at any of the temperatures evaluated in the current study. When assessed *in vivo*, inactivation of *L. innocua* was significantly influenced by temperature (p<0.001), processing time (p<0.001), ultrasounds (p<0.05), and the combination of all factors (p<0.05). For example, reductions in the population of *L. innocua* after thermosonication at 55 ºC for 5 min were 5.2-log cfu/g. These were higher when compared to those observed after thermal processing alone at the same temperature, calculated as 3.2-log cfu/g. Thermosonication for 5 min at 55 ºC also led to higher *L. innocua* inactivation when compared to chlorine at 200 ppm (pH 6.5) for 2 min (3.5-log cfu/g). Longer processing times led to increased inactivation of *L. innocua* (p<0.001). However, these conditions resulted in reduced overall quality (loss of texture, colour, and visual appearance), leading to strawberries that could still be used for the development of processed products but that could not be sold as fresh produce. The lethal effect of temperature combined with ultrasounds on the native microbiota of the strawberries was higher when compared to that of sonication or thermal processing alone (p<0.05). These results were also observed on strawberries when used as an ingredient in the manufacture of a fruit-based juice [3]. Thermosonication also led to an increased bioaccessibility of polyphenols after a simulated gastrointestinal digestion.

CONCLUSIONS: Thermosonication can be used as a pre-treatment to reduce the content of *L. innocua* and the native microbiota in strawberries. The antimicrobial effects of thermosonication depend on several factors, which include the food matrix and the studied microorganism.

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Use of a seaweed-enriched kefir whey in the development of edible food coatings

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The growth of the food industry and of its distribution chains demands steady innovation. The introduction of active and biodegradable packaging is an effective strategy to meet these demands. [1] A substantial amount of research in food biotechnology involves the discovery and characterization of new sources of natural bioactive materials. Among these, marine resources remain largely unexplored as sources of bioactive compounds. Alternatively, some researchers have explored less conventional resources, in the form of yeast and moulds used in food processing and transformation. Kefir is a dairy product that is obtained by the fermentation of milk through use of kefir grains. These are, in turn, a complex mixture of lactic acid bacteria and yeast in symbiotic coexistence. Kefir has long been regarded as a probiotic food, yet over recent years, it has also been cited as a source of bioactivities, including antimicrobial, antioxidant, anti-inflammatory, and antitumoral. These activities have, in part, been attributed to an important set of hydrophilic molecules, including peptides and polysaccharides. [2, 3, 4]

The shelf-life enhancing effects of Kefir whey, combined with Gracilaria gracilis extracts, were evaluated as components of edible food coatings used in whole strawberries, a highly perishable fruit.

The strawberries were coated in kefir whey solutions with and without seaweed extract and were then stored in room temperature for a period of four days. Solutions of commercial algal polysaccharides were used as controls. Total microbial counts and yeast plus mould counts were used to monitor microbial degradation throughout the storage period. Daily sensory analysis trials (triangle tests) were used to evaluate production of off-flavour compounds, as well as the impact of the use of coatings and extracts.

The coatings failed to extend the shelf-life of the strawberries, with all samples deemed inacceptable for consumption upon the fourth day of storage, either through development of moulds in uncoated/control coating strawberries or by natural degradation of texture. However, microbial counts revealed no growth of filamentous fungi in kefir whey-coated samples, and the triangle tests showed no significant differences between coated and uncoated strawberries according to the test panel.
The results show a potential antifungal activity in kefir whey potent enough to be useable as a basis in edible coatings. Further studies will further examine this potential and widen the use of kefir whey coatings to different food types.

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Variability of L-asparagine level in sea buckthorn berries and acrylamide formation in novel cereal products

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L-asparagine amino acid is a key determinant of acrylamide formation during thermal processing of foods. Incorporation of sea buckthorn in novel cereal products besides unambiguous positive aspects undesirably increases the risk of acrylamide formation due to the higher content of L-asparagine. L-asparagine content in sea buckthorn berries *(Hippophae rhamnoides L.)* planted in Slovakia ranged from 1600 mg/kg to 2530 mg/kg. The highest L-asparagine content was determined in special dry sea buckthorn by-products consisted of berry skins usually utilized as an excellent natural colorant with high content of carotenoids.

Incorporation of sea buckthorn puree into high moisture cereal-based models (cakes and muffins) only slightly increased acrylamide content. In all tested cereal-based samples with sea buckthorn puree addition, the acrylamide content was less than 150 µg/kg and did not exceed the required benchmark level. On the other hand, the significant increase of acrylamide was observed in dry model cereal-based systems (biscuits) where an incorporation of dry sea buckthorn by-products to wheat or spelt composite flour mixes led to acrylamide formation up to 560 µg/kg. Therefore, the addition of dry sea buckthorn in composite flour mixes should be limited to 10 – 20 % which is convenient also from organoleptic acceptability. In order to eliminate risk of acrylamide formation, combination of dry sea buckthorn with low asparagine cereals such as rice, corn and buckwheat can be recommended. However, it should be borne in mind that combination with legumes such as pea, peanut, soy, chickpea and hemp increases risk of acrylamide formation in novel cereal-based products due to higher amino acids and especially L-asparagine content in comparison to wheat.

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Matrix effect in multi-pesticide residues analysis: the complexity in tea commodities

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Tea is the second drink after water and has become a popular drink due to some health benefits. However, pesticides are unavoidable used during tea cultivation due to monoculture practice and monoclonal varieties in areas with high humidity and temperature with parasites. Maximum residue limits (MRLs) for pesticides have been established to control the tea quality and protect consumers' health in many countries, especially in Europe. QuEChERS (quick, easy, cheap, efficient, rugged and safe) methods have been widely used for pesticide analysis in food[1,2]. However, matrix effect (ME) is a serious problem in applying this method, especially in the case of very complex matrices as tea commodities. Ion suppression and enhancement are two ME causes inducing lower or higher, respectively, pesticide concentrations obtained by analysis procedure than true concentrations. ME can be compensated by: (i) dilution; (ii) use of standard addition; (iii) addition of internal isotope standards; (iv) matrix-matched calibration; and (v) improvement of the sample preparation to eliminate or reduce the interferences[3,4]. Dilution, which is a simple, effective method for reducing ME, requires a high sensitivity of the analytical equipment to reach the pesticide MRLs in food. Standard addition is not suitable for routine analysis. Internal isotope standards are very expensive and usually not available for hundreds of substances. Matrix-matched calibration would be the best way to overcome the ME phenomenon and give high accuracy results for multi-pesticide-residues analysis. However, many matrix-matched calibration curves need to be applied, since the obtained ME depends not only on the variety and origin of teas but also on the process to produce different kinds of tea[5]. Therefore, the QuEChERS method has to be improved for simultaneously multi-pesticides-residues analyses with high recovery and sensitivity, and low ME by combining the sample preparation/purification. In our study, we obtained good ME results for analysing 400 pesticide residues in green tea by combining QuEChERS extraction and mix-mode solid phase extraction clean-up procedure (including C18, GCB and PSA sorbent). We showed that 225 compounds (ME in the range of ± 20%) can be quantified by calibration curves in solvent without using matrix-matched calibration curves (Table 1): our ME results being significantly higher than the ones of other results[6,7] with 154 compounds (accounting 77% for LC-MS/MS) and 71 compounds (accounting 35.5% for GC-MS/MS). Further work is in progress to test our method to other types of teas such as black, oolong and white teas.

Table 1. Evaluating matrix effect on two methods

ME (%)	Number of pesticides (% proportion)	
	UPLC-MS/MS	GC-MS/MS
< (–50)	12 (6.0)	3 (1.5)
(–50) – (–20)	33 (16.5)	4 (2.0)
(–20) – (+20)	154 (77.0)	71 (35.5)
(+20) – (+50)	1 (0.5)	38 (19.0)
> (+50)	0 (0)	84 (42.0)

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I.FILM: Multifunctional films for application in active and smart packages

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Due to high perishability, poultry products are kept in refrigerated storage within protective packaging and modified atmosphere. Lipid oxidation, while not often regarded as a limiting factor in the shelf-life of these products, becomes a focal point when other deteriorative effects are suppressed. [2]
Poultry is particularly susceptible to lipid oxidation due to a lipid profile rich in polyunsaturated fatty acids. This means it is still a relatively perishable product, with which a small gain in shelf-life becomes highly profitable. Innovation in the packaging systems themselves can prove to be an effective and profitable way to achieve this, as recent advances in active and smart packaging have led to believe. The incorporation of antioxidant and antimicrobial agents in packaging films has demonstrated reliable increases in product stability. However, the synthetic source of these agents and/or their extraction procedures often make them undesirable from an environmental and economic perspective. [1, 2, 4]

Widely available seaweed biomass presents itself as an ideal source of both natural polymers, having a wide range of versatile and functional structural polysaccharides, and a unique range of bioactive compounds with antioxidant and antimicrobial potential. Thus, the incorporation of marine bioactives in food packaging as a means of extending shelf-life presents itself as a novel and effective method of remedying current difficulties in the food industry, while fitting within modern circular economy systems and adding value to marine resources. [1, 3, 4, 5]

Project I.FILM attempts to fulfil this vision through the development of thermoplastic food coatings enriched with marine bioactives from readily available aquaculture macroalgae.

Hydroethanolic extracts of red macroalgae *Gracilaria gracilis* and *Porphyra dioica* were evaluated for their antioxidant potential (DPPH and FRAP assays) and phenolic content, as part of the preliminary assays for the selection of algal biomass enriching the thermoplastic films. Upon statistical optimization of the extraction process using Response Surface Methodology (RSM), the extracts were processed through electrospinning into a nanofiber coating, using high molecular weight PEO (polyethylene oxide) as the base polymer. Bioactive stability of the electrospun material was then evaluated through the same antioxidant assays.

The project further encompasses the determination of effective shelf-life gains from the use of the new active films, as well as the study of compound migration from the nanofiber-enriched films, the effect of electrospinning conditions in the mechanical and bioactive quality of the film, and the optimization of film design for minimal production costs.

Depletion of methylene blue from muscles of rainbow trout (Oncorhynchus mykiss)

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Methylene blue is a phenotiazine dye which has been used worldwide as fungicide and ectoparasicide in aquaculture [1]. Its metabolites are azur A (AZA), azur B (AZB), azur C (AZC) and thionine (TH) [2]. MB induces carcinogenic effects and according to the International Agency for Research on Cancer (IRAC) it was classified to the third group of toxicity, i.e. not classifiable as to carcinogenicity to humans. [3,4]. Thus, its use in the aquaculture products intended for consumption is not allowed and the presence of its residues in fish muscles after potential illegal use should be controlled [5].
So far, the study on metabolism and depletion of methylene blue in fish has been conducted only on catfish [6] and no data have been reported for rainbow trout. Therefore, the aim of the study was to evaluate the depletion of MB from rainbow trout muscle after a therapeutic bath.

The trout (150) were exposed to MB at a nominal concentration of 2 mg/l bath water for 24 h as recommended by Noga [1]. After the treatment, the fish were transferred into a tank with clean water. At regular time intervals, groups of 10 fish were collected, euthanized and killed. Moreover, 10 trout were euthanized and killed before the start of dye administration and used as the control group. Muscle tissues of each group of animals were then analysed for quantitative MB, AZA, AZB, AZC and TH determination by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The method applied was validated according to the guidelines laid down by the European Decision 657/2002/EC [7].

During waterborne exposure MB was absorbed in muscles and converted into AZB which were slowly eliminated from the tissues. AZA and AZC were also found in muscles but in lower concentrations. This shows that parent compound MB itself is the main residue in rainbow trout muscles.

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ORAL COMMUNICATIONS

Food Sustainability

Chemical composition and bioactivities of Juçara fruit bio-residues, a promising source of valuable molecules

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Euterpe edulis Martius, popularly known as Juçara, is a native tree of the Atlantic Rainforest found predominantly in the states of the southern and southeastern regions of Brazil. Juçara fruit is a globose berry that, when ripe, acquires a dark purple shade and sweet pleasant taste. The industrial production of Juçara fruit pulp generates solid residues (peel) which are usually discarded [1]. The aim of the present work was to perform an unprecedented in-depth study on the bioactive components profile of *E. edulis* fruit peel. The nutritional composition of this material was estimated and its hydroethanolic extract was characterized in terms of phenolic compounds, besides antioxidant and antibacterial potential. Finally, the hepatotoxicity of the extract was assessed. A total of nineteen phenolic compounds was identified in Juçara peel. Seventeen were non-anthocyanin molecules: two phenolic acids, namely caffeic acid and a ferulic acid derivative; four flavanonols, corresponding to three dihydroquercetin (taxifolin) and one dihydrokaempferol (aromadendrin) glycoside derivatives; six flavones, assigned as apigenin *C*-glycoside derivatives; and five flavonols, among which quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, and isorhamnetin-3-*O*-rutinoside. The major components of the peel extract were the anthocyanins cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-glucoside (5.32 and 6.23 mg/g of extract, respectively), which together accounted for more than 87% of the extract's total phenolic content, which corroborates literature data [2, 3]. Anthocyanins are amidst the main compounds related to the great free radical-scavenging capacity of Juçara fruit, whereas a significant positive correlation with its general antioxidant capacity was observed (Shultz et al., 2016). The herein studied fruit peel extract presented expressive values of antioxidant capacity, assessed by five distinct methods, namely (1) oxidative haemolysis inhibition assay (OxHLIA), (2) inhibition of the production of thiobarbituric acid reactive substances (TBARS); (3) reduction of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH); (4) reduction of the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonate) cation (ABTS), and (5) reduction power of the ferric ion (FRAP). Furthermore, the results obtained for antioxidant activity were more expressive than the ones verified by other authors for Juçara residues [1]. The evaluated *E. edulis* extract showed no toxicity against a non-tumour liver primary culture PLP2, at the highest concentration assessed (400 µg/mL). The inhibitory activity displayed by the extract against both Gram-positive (*Enterococcus faecalis*, *Listeria monocytogenes*, and Methicillin-resistant *Staphylococcus aureus* - MRSA) and Gram-negative (*Escherichia coli*, *Klebsiella pneumonia*, *Morganella morganii*, and *Pseudomonas aeruginosa*) bacteria indicates the presence of an extensive spectrum of phytochemical constituents with antibiotic potential. Indeed, the extract was more effective than the antibiotic ampicillin against *M. morganii* and *P. aeruginosa.* Therefore, Juçara fruit residues could be used to produce high added-value food ingredients, both colorants and preservatives, following the circular bioeconomy concept and stimulating the Juçara production chain.

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Antimicrobial potential of essential oils from agro-industrial by-products as possible feed ingredients

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Thyme, oregano, and winter savory are worldwide consumed aromatic plants whose essential oils (EOs) are used as spices and preservatives by food industry. Only the leaves are marketed, generating a large amount of stems as by-products. However, it is possible that the EOs present in these by-products represent a source of compounds with antimicrobial activity, as observed for the leaves.

A wide range of antibiotics are used worldwide within the poultry industry for therapeutic, prophylactic, and as growth promoters [1]. Their extensive use is on the basis of the occurrence of bacterial resistance to the most widely used antimicrobials [2]. Under this scenario, it is imperative the development of alternative substances and strategies for animal growth promotion and disease prevention. Phytobiotics, highly available in agro-food by-products, are candidates, as they have been investigated as growth promoters in poultry industry [3]. The present work evaluates the antimicrobial activity of EOs from thyme, oregano, and winter savory by-products as possible ingredients in broiler diets.

The EOs from the stems of thyme "Bela-luz" (*Thymus mastichina*), oregano (*Origanum vulgare*) and winter savory (*Satureja montana*) were obtained by hydrodistillation (≈120 min) with convective heating, and by solvent-free microwave extraction (16±1 min) of soaked in water and drained stems. The EOs obtained by different extraction methodologies and harvest had similar chemical composition. A total of 37 compounds were identified in thyme and oregano, mainly monoterpenoids, with carvacrol (17%) and thymol (1% and 11% in thyme and oregano, respectively) as the major compounds on both species. Sesquiterpenoids account also for about 10-24%. In winter savory, a total of 13 compounds were identified, accounting the monoterpenoids with 84.4–97.6% and sequiterpenoids with 0.3–0.5%, being carvacrol the highest component (825–950 µg/mg).

The three EOs showed antimicrobial activity, individually evaluated against *Escherichia coli* ATCC 25922, *Salmonella enterica* sv Anatum SF2 and *Staphylococcus aureus* ATCC 6538 using an agar disc diffusion method and broth microdilution assay. The winter savory showed the highest antimicrobial activity, associated to its higher carvacrol concentration.

In conclusion, the winter savory, thyme, and oregano by-products have potential to be incorporated in animal feed as antimicrobials against the main poultry's infectious species.

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Enhancing Proteins extraction from *Moringa Oleifera* leaves: From conventional methods to a fully integrate process

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Increasing attention is given to alternative proteins from plant origin to be used in human diet. *Moringa Oleifera* (fam. Moringaceae), is a perennial foliaged tree, widely cultivated in tropical and subtropical countries due to its high adaptability to climatic conditions and dry soils [1]. *Moringa Oleifera* leaves (MOL) account for numerous tones of biomass per year, and has potential to be a good protein source, since the protein content ranges between 22.2% to 31.4%. Moreover, it has a good balance of essential amino acids and biopeptides [2,3]. Nevertheless, the anti-nutritional factors in MOL may hinder efficient extraction, absorption and digestion of protein. Additionally, they can decrease protein bioavailability and nutritional status [4,5]. In this context, the novelty of this work is the separation of MOL by the development of an integrated process for simultaneous extraction of protein fractionation from the other components of leaves, such as phenolic, fibre and lipids. For that purpose, carbohydrate-hydrolyzing enzyme was use and selective separation of proteins was carried out.

Protein extraction from MOL was achieved by the utilization of viscozyme L and cellulase enzymes along with recovery of different bioactive fractions (phenolic, lipid and fibre fractions). The enzymatic treatment was used for the cell wall disruption, in order to dissociate proteins from polysaccharides and more intracellular bioactive fractions. This method was more advantageous than the use of traditional alkaline treatment, with the increase of protein yield from 21% (for alkaline treatment) to more than 70% with enzyme application. The optimum conditions were obtained using defatted MOL and a sequential treatment with viscozyme L (30 FBG / g) for 1h at 40 °C, precipitation with ethanol (4:1, v:v) overnight at room temperature, and further use of cellulose (2%) for 1h at 40 °C.

A fully integrated process for separation of lipid, phenolics, fibre and protein fractions from MOL was optimized. This innovative environmentally clean process allows total valorisation of these bioactive fractions that may have several uses in food and pharmaceutical fields.

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Agrocybe cylindracea bio-residues: a sustainable source of ergosterol-rich bioactive extracts

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The world production of edible mushrooms has increased more than 30-fold since 1978 and, on average, each customer consumes 5 kg of mushrooms per year [1]. Depending on the dimension of the mushroom industry, the amount of obtained by-products may range from 20 to 35% in weight of fresh mushroom [2]. Indeed, 38% of the 90 million tonnes of food waste produced by the European Union every year has its origin in the food manufacturing sector [3]. However, there are several strategies of transforming these wastes into high value-added products based on the cascade use principle, and this is exactly what the MicoBioExtract project aims to perform. Thus, the bio-residues of a popular edible mushroom in southern Europe, *Agrocybe cylindracea*, were studied as a sustainable source of bioactive extracts [4].

Ergosterol has been reported to be one of the most important compounds, contributing to the health-promoting benefits, associated with mushrooms' consumption [2]. Its extraction was performed using a heat-assisted technique and applying the response surface methodology, in order to optimize the combined effects of the variables time, temperature and solvent percentage, using a circumscribed central composite design with 16 independent combinations and 4 replicated centre points. At the optimum condition predicted by the model, the bioactivity of the extract was tested, evaluating the antioxidant (TBARS assay) and cytotoxic (in a porcine liver primary cell culture, PLP2) activities.

The results obtained from the optimization study showed a significant interaction between temperature and extraction time, with an 8.24% extraction yield. The global optimum condition for ergosterol extraction predicted by the model was 150 min at 90 °C, with 329 mg of ergosterol per 100 g of dry weight sample. Regarding the bioactive potential, namely the antioxidant activity, this extract was capable of preventing the formation of malondialdehyde, a secondary product of lipid peroxidation in the TBARS assay. Concerning the cytotoxicity of the extracts against the PLP2 cell line, the results showed no significant cytotoxic effect, with GI_{50} values higher than 400 µg/mL.

Thus, the extraction of molecules with a high nutritional and bioactive value from mushroom bio-residues and the goal to incorporate them in functional foods and nutraceuticals could boost the circular bio-economy, and help developing strategies towards promoting sustainability.

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Tailored farmed fish iodine and selenium fortification with naturally enriched diets: gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) as case studies

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One third of the world's population suffers from malnutrition, particularly evidencing deficiencies in essential elements like selenium and iodine. Despite the benefits of seafood consumption are widely recognized, when it comes to farmed seafood many questions regarding their nutritional quality and safety still persist among the general population. Hence, the potential to develop tailor-made fortified farmed seafood products with adequate levels of essential nutrients can be an excellent tool not only to upgrade their quality, but also to increase consumers' confidence in these products [1,2]. In this context, the present study aimed to evaluate the use of naturally enriched fish diets (iodine-rich macroalgae and selenised-yeast) as an efficient tool towards iodine and selenium fortification in farmed gilthead seabream (*S. aurata*) and common carp (*C. carpio*) fillets.

A control commercial diet and three experimental diets incorporating various blends of macroalgae and selenised-yeast were formulated for each species. Fish were feed ad libitum, during three months, mimicking the end of the production stage (i.e. just before reaching market size; 350-400g). Each diet was tested in triplicate tanks (n = 76 fish/diet) and fish were slaughtered 48h following the last meal, by immersion in chilled seawater, as is typical commercial practice. Fish skinless fillets were collected (n = 3 pools of 5 fish each) and iodine and selenium contents were determined by ICP-MS [3,4].

In line with previous studies [5], the fillets of fish fed with the highest iodine and selenium supplemented diets showed a significant enhancement in the contents of both elements compared to the control diet, i.e. increased Se (98% in seabream and 41%in carp) and I (37% in seabream and more than 100% in carp). Hence, the consumption of fortified seabream and carp fillets (200g) represented an increase in the adequate intake (AI) of iodine and selenium for adult consumers. Indeed, fortified fillets represented 12% (seabream) and 25% (carp) of iodine AI, and more than 100% (seabream) and 38% (carp) of selenium AI, whereas non-fortified fillets (CTR) represented 9% (seabream) and 2% (carp) of iodine AI, and 52% (seabream) and 27% (carp) of selenium AI[6,7]. This work revealed the importance of fortified fish products, towards the sustainable, secure and high-quality production of farmed seafood in Europe to meet consumers' nutritional deficiencies and needs.

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At-line boar taint classification by means of Rapid Evaporative Ionisation Mass Spectrometry (REIMS)

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Increasing awareness of animal welfare has led to a European incentive to ban the surgical castration of piglets. A valid alternative for castration is the rearing of entire male pigs, but this allows the (re)occurrence of boar taint, an off-odour in meat from entire boars [1]. Hence, due to adverse consumer reactions to pork with boar taint, the rearing of entire boars requires valid boar taint mitigation strategies. However, the introduction of Rapid Evaporative Ionisation MS (REIMS) offers compelling perspectives for the rapid as well as accurate at-line detection of boar taint by significantly reducing analysis time and workload, yet enhancing research output and efficiency [2].

In this study, REIMS was used as a direct analysis technique to train predictive models for identification of boar taint above the odour threshold (based on sensory (soldering iron method) as well as chemical analysis (UHPLC-HRMS analysis of indole, skatole and androstenone levels) [3]. Adipose tissue was sampled using a prototype bipolar handheld sampling device connected directly to a Xevo G2-XS Q-TOF system equipped with REIMS source (Fig. 1).

The results demonstrate that untargeted mass spectrometric profiling in negative ionisation mode enables the construction of predictive models using LiveID, AMX and Simca (Q^2 = 0.547, R^2Y = 0.652 and p = 0) for the classification of carcasses according to boar taint status based on alterations in lipid profiles. As REIMS eliminates sample pre-treatment with analysis taking < 10 seconds, it offers significant potential as the first technique enabling accurate *in-situ* detection of boar taint. REIMS is a promising and highly innovative tool for several types of food quality and safety applications, furthermore allowing us to move state-of-the-art equipment and applications from bench to production site.

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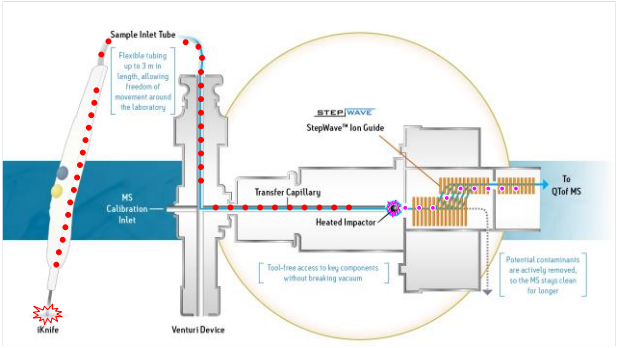

Fig.1. Schematic overview of the REIMS platform.

Anthocyanins Thermostability Modulation Through the Fortification with Pectic Polysaccharides Extracts

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A major trend in the food industry is the development of convenient fruit and vegetable derived food products using naturally sourced ingredients [1]. Anthocyanins are the largest and most important group of water-soluble pigments, responsible for the bright colours of flowers, fruits and vegetables [2]. Due to their colours and beneficial health effects, there has been a growing industrial interest in these pigments, particularly as food colourants [3]. However, anthocyanin typically degrades during thermal processing and storage, affecting their content in the final food product, colour quality and the nutritional properties [4, 5].

Although the clear evidences of anthocyanins-pectic polysaccharides interactions and despite the potential significance of this binding in food chemistry and nutrition [6-11], detailed information regarding the possibility of using pectic polysaccharides as anthocyanins thermo-stabilizers for food applications is lacking.

To elucidate the effect of cell-wall polysaccharides on anthocyanins thermostability, pectic polysaccharides rich fractions were isolated and fractionated from white grape skins alcohol-insoluble residue into water soluble (WSP), chelator soluble (CSP) and acid soluble (ASP) pectic polysaccharides fractions. These fractions were incubated with malvidin-3-*O*-glucoside in aqueous solution at pH 3.5 and subjected to a thermal treatment at three temperatures (60, 80 and 100º C). Thermostability was evaluated assessing anthocyanins residual content by means of HPLC-DAD. Calculation of the degradation parameters such as reaction rate constant (k), half-lives ($t_{1/2}$) and activation energy was conducted (E_E), following a first-order reaction model. Overall, fortification with pectic polysaccharides showed an improved colour stability than malvidin-3-*O*-glucoside alone at pH 3.5. Anthocyanins and some of their degradation products were monitored and quantified using HPLC/ESI-MS.

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Simulated gastrointestinal digestion increases the antioxidant activity of *Porphyra dioica*

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Porphyra dioica is a valuable commercially red seaweed commonly known by "nori". This study aimed to characterised the nutritional composition of *P. dioica* blades produced in an integrated multitrophic aquaculture system. Furthermore, the influence of simulated gastrointestinal digestion (SGID) on antioxidant activity was evaluated.

Blades were harvested and dried (air tunnel ventilation, 25 ºC). Proximate composition was determined using official methods [1] as follows: ash (incineration at 550 ºC); crude protein (Kjeldahl Nitrogen estimation); total fat (Soxhlet extraction) and fibre (combined enzymatic and gravimetric methods). Carbohydrate content was estimated by difference. Total phenolic content was determined by the Folin-Ciocalteau method. Additionally, blades were submitted to *in vitro* SGID (using pepsin, 90 min at 37 ºC and Corolase PP, 180 min at 37 ºC). The enzyme:substrate used was based on the previously determined protein content. The antioxidant activity of the freeze-dried hydrolysates and respective controls (sample without enzymatic treatment) was determined using a range of *in vitro* assays: ferric reducing activity power (FRAP), oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging and the trolox equivalent antioxidant capacity (TEAC) using AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) as radical generator ($ABTS^{\bullet+}$, 2,2-azino-bis(ethylbenzo-thiazoline6-sulfonic acid) [2].

On a dry weight (dw) basis the blades contained 33.7±0.8% of total fibre, 26.2±0.5% protein;16.8±0.2% of ash and 0.4±0.1% lipids. Available carbohydrates represented 22.9% dw. The total phenolic content was 2.93±0.14 mg gallic acid equivalents/g dw.

Apart from the $DPPH^{\bullet}$ scavenging activity, the control blades generally had low antioxidant activity in the *in vitro* assays (Table 1), which significantly increased following SGID. These results highlight the importance of using different methods to test for antioxidant activity, and also showed that SGID treatment promoted the release of bioactive peptides and/or other compounds (e.g., phenolics, reducing amino acids) capable of scavenging $ABTS^{\bullet+}$ (TEAC) and peroxyl radicals ($ROO^{\bullet}$) (ORAC).

Table 1. Antioxidant activity (µmol trolox equivalents/g dry sample) of *P. dioica* young blades.[1]

	Control	SGID Hydrolysate
FRAP	10.1±1.4 [a]	15.6±1.3 [b]
ORAC	329.9±88.1[a]	2010.0±135.6[b]
$DPPH^{\bullet}$	6.0±1.7[a]	9.9±0.4[a]
TEAC	65.0±7.8a	230.7±14.6[b]

[1] Results are presented as mean ± standard deviation (n=3). Different letters in the same line represent significant differences between the control and the SGID hydrolysate (p<0.05).

In summary, young *P. dioica* blades are a good source of dietary fibre, protein and minerals. Additionally, they may be a promising source of bioactive compounds, including phenolics and protein bioactive peptides. Overall, while more studies are needed to validate these biological effects *in vivo*, the results show that after digestion the antioxidant activity of blades may be significantly enhanced.

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POSTERS

Food composition and Authenticity

Quality assessment of rapeseed oil during deep-frying process using electronic nose with electrochemical sensor array

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Electronic noses have been used for years to evaluate the quality of food, mainly due to their strengths such as short analysis time, relatively low price and ease of measurement. There are many examples of the use of electronic noses in food evaluation, including assessment of the quality of vegetable oils. [1]

Currently, the most prevalent sensors in electronic noses are semiconductor sensors (mainly MOS type). Despite their advantages, such as low price and versatility, they suffer from such problems as high energy consumption, sensor drift or strong dependence of the response signal on relative humidity which limits their use in the area of food analysis. Therefore, it is necessary to look for alternative solutions that will overcome these limitations.

The developed prototype of the electronic nose is equipped with four interchangeable sensor modules working independently, so it is possible to tailor its operation to the dedicated application (See Fig. 1). In the electronic nose we implement modern, commercial screen-printed electrochemical sensors. Using this device it is possible to analyze a single sample in less than five minutes. [2]

The prototype was used in the study to determine the quality of rapeseed oil during deep-frying process of potato (*Solanum tuberosum L*.) slices. Experiment were carried out both in off-line and on-line modes. The obtained data was processed using supervised and unsupervised data analysis tools.

The studies were complemented with identification of volatile compounds and measurement of thermal stability of the oil samples collected during frying. Using the prototype it is possible to determine the quality of oil, thus this procedure can possibly be used in food processing industry.

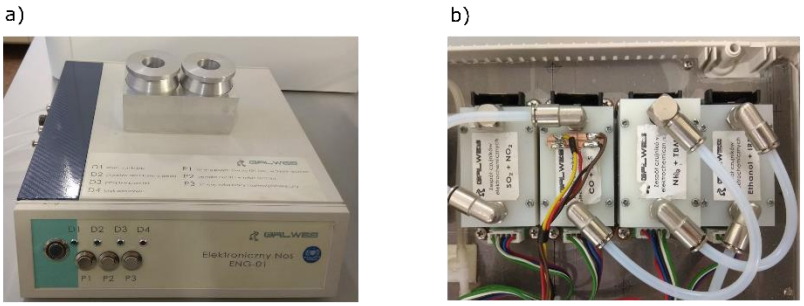

Fig.1. a) The view of an electronic nose prototype; b) The removable sensor modules

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Assessing the varietal origin of Portuguese Olive Oils by NMR spectroscopy and multivariate statistical analysis

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Olive oil is one of the major constituents of the Mediterranean Diet showing beneficial effects for human health. Extra virgin olive oil (EVOO) has a relatively higher price on the market, and thus it is becoming mandatory to establish its authenticity. Moreover, in the last years, much attention has been given to fraudulent practices associated with olive oil traceability focused with special emphasis on the botanical origin due to the recent introduction in the market of high-quality monovarietal olive oils [1].

NMR spectroscopy in combination with multivariate statistical analysis seems to be a promising and powerful technique for discriminating olive oils geographic and botanical origins. In fact, ^{1}H NMR and ^{13}C NMR spectroscopy are nowadays consolidated techniques that can be successfully applied for the identification of the geographical and botanical origin of Olive Oils [2].

In this work, we will discuss the use of NMR spectroscopy in combination with multivariate statistical analysis to discriminate Portuguese Olive Oils in terms of botanical origin. For these studies, ^{1}H NMR and ^{13}C NMR spectroscopy were used to assess the triacylglycerol fraction of these olive oils. Furthermore, statistical tools were used to verify the suitability of these spectroscopic techniques for the discrimination of the botanical origin of the Portuguese Olive Oils under study.

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Identifying the geographical origin of Serra da Estrela PDO cheeses using fatty acids profiles

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Serra da Estrela is a traditional Portuguese cheese with a Protected Designation of Origin (PDO) certification. This cheese is produced from raw ewe's milk from "Churra Mondegueira" and "Bordaleira" Portuguese autochthonous breeds and coagulated using wild thistle flower (*Cynara cardunculus* L.), and its production is geographically limited. Serra da Estrela is the most known and popular Portuguese cheese and is appreciated worldwide, being preferentially consumed as a soft cheese, with an average maturation of 30-45 days, although some consumers prefer to consume it as a hard cheese after at least 6 months of storage [1]. Due to its social and agroeconomic relevance, Serra da Estrela cheese is prone to geographical origin adulterations. The present work aims to verify if the fatty acids (FA) profile could be used as a geographical origin biomarker. The results showed that, although a similar FA profile (23 individual fatty acids identified, being the most abundant ones: $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1n9c}$, $C_{18:2n6t}$, $C_{18:2n6c}$ and $C_{18:3n3}$) could be established for all cheeses, regardless the producer, geographical origin and production date, the overall profile could be used for discriminating the cheeses according to their geographical origin (5 municipalities within the PDO region). A linear discriminant analysis (LDA) with the simulated annealing (SA) algorithm enabled establishing a classification model that was able to correctly classify 96% of the original grouped samples (**Fig.1**) and had a predictive sensitivity of 88% (leave-one-out cross-validation). So, FA profile could be used as a geographical origin authentication tool, providing the consumer a guarantee regarding this high-value and appreciated food.

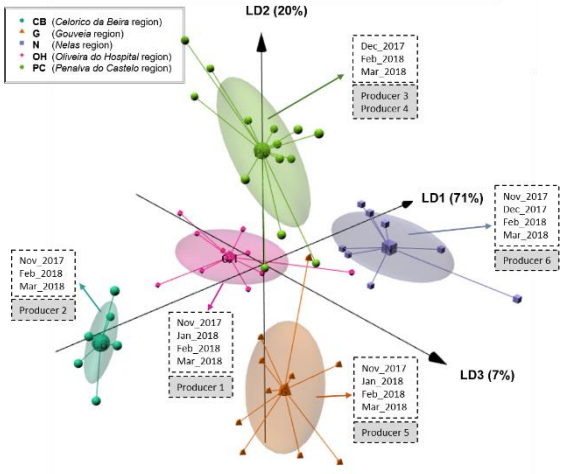

Fig.1. LDA-SA discrimination of Serra da Estrela PDO cheeses by geographical origin.

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Development of a UPLC-MS/MS method for establishing Serra da Estrela's free amino acids profile

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Serra da Estrela cheese is a high-value and widely appreciated Portuguese cheese, which as a Protected Designation of Origin recognition, being its production legally regulated. The protein portion of foods, as well as its amino acids composition (namely the essential amino acids), represents a fundamental role on the nutritional and technological value of cheese, influencing greatly its flavour [1,2]. A UPLC–MS/MS, comprising a Dionex Ultimate 3000 UPLC instrument equipped with a diode-array detector coupled to a mass detector, was used to establish the free amino acids profile of Serra da Estrela cheeses. The amino acids chromatographic separation was accomplished using a U-VDSpher PUR C18-E column (100 mm×2.0 mm id, 1.8 µm column) at 40 °C. The MS detection was performed in positive mode by multiple reaction monitoring (MRM) using a Linear Ion Trap LTQ XL mass spectrometer (equipped with an ESI source. Mass spectra were acquired by full range acquisition covering 100–1500 *m/z*. The collision energy used varied from 14 to 30 (arbitrary units) depending on amino acid. Cheese, from 6 certified producers, were acquired after 45 days of ripening, being the amino acids extracted using a water-acetonitrile solution, to which N-acetyl-L-tyrosine was added (as an internal standard). In total, 21 amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, histidine, lysine, serine, threonine, valine, methionine, 4-hydroxy-proline, isoleucine, leucine, phenylalanine, tryptophan and tyrosine) were assessed (**Table 1**) and detected in the cheese samples, being the results in accordance with previous works [1]. The chromatographic method developed showed to be very accurate and allowed overcome experimental drawbacks arisen with conventional liquid chromatography.

Table 1. Free amino acids detection by UPLC–MS/MS

Amino acid	Quantification transition (m/z)	Confirmatory transition (*m/z*)	Amino acid	Quantification transition (m/z)	Confirmatory transition (*m/z*)
Histidine	156	137, 111, 109, 94	**4-Hydroxy-Proline**	132	85
Lysine	147	130, 129, 100	**Isoleucine**	132	120, 114, 104, 86, 85, 71, 68
Glutamine	147	129, 100, 83	**Leucine**	132	120, 114, 104, 86, 85, 71, 68
Glutamic acid	148	130, 129, 101, 83	**Asparagine**	133	115, 112, 104, 87, 89, 85
Serine	106	88, 87, 85, 59	**Arginine**	175	157, 140, 130, 115, 111, 97
Alanine	90	68, 61	**Phenylalanine**	166	148, 130, 119
Glycine	76	75, 47, 29	**Tryptophan**	205	187, 159, 132
Threonine	120	101, 99, 83, 73, 71, 55	**Cysteine**	121	98, 97, 75
Aspartic acid	134	115, 87,86, 73	**Tyrosine**	182	164, 135
Valine	117	100, 90, 71	**Cystine**	241	224, 14, 177, 168, 93, 151
Methionine	150	132, 103, 101, 55			

Acknowledgments: This research was financially supported by Associate Laboratory LSRE-LCM-UID/EQU/50020/2019, strategic funding UID/BIO/04469/2019-CEB and BioTecNorte operation (NORTE-01-0145-FEDER-000004), strategic project PEst-OE/AGR/UI0690/2014–CIMO and by Project 02/SAICT/ 2016/23290-QCLASSE, funded by national funds through FCT/MCTES (PIDDAC).

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Influence of harvesting time on biogenic amines profile in Italian hempseed

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Over the recent years, several consumers moved to healthy and natural lifestyles. The main contribution to these lifestyles is given by the change in diet. Consumers are looking for natural foods, rich in nutrients and with a low environmental impact. Hemp seeds are protagonists of this change. It comes from seeds of selected varieties of the Cannabis Sativa L plant. Canapa Sativa is an ancient industrial plant with multiple uses. This plant has been used in the past to produce cordages, fabrics, paper, cosmetics and is free of the psychoactive substance tetra-hydro-cannabinol THC (THC <0.2%, EC Reg. No 1673/2000; EC Reg. No 73 / 2009). Furthermore, hemp seeds have an optimal nutritional profile: fair amount of proteins with high biological value, ideal ratio of unsaturated fatty acids ω3:ω6, micronutrients and antioxidants presence. Nowadays, the food involves of Canapa Sativa is growing up. The hemp-food market is steadily increasing, thanks to its high nutritional value. The aim of this study was to characterize some hempseeds cultivated in Italy, evaluating the profile of 8 biogenic amines (histamine, serotonin, spermine, spermidine, putrescine, β-phenylethylamine, cadaverine, tyramine). Biogenic amines are widely proposed as a marker to evaluate the quality, safety and geographic or botanical origin of the raw materials. Biogenic amines are molecules derived from enzyme or microorganism activity, found in several foods and beverages. They come from amino acids decarboxylation and can cause several effects in human organism like food poisonings, scombroid syndrome and the cheese crisis. For these reasons, some biogenic amines as histamine and tyramine are usually monitored in food to ensure the hygienic-sanitary safety. In this study a reverse phase liquid chromatography method coupled with a spectrofluorimetric detector (RP-HPLC-FD) and pre-column derivatization was used. Sampling was carried out by considering different harvesting time, cultivars and storage methods of seeds. The results show that the BAs profile was influenced by cultivar and harvesting time, with a total concentration <300 mg / kg in all samples (Fig. 1). Moreover, a multivariate statistical (chemometric) treatment of data matrix was performed. An exploratory data analysis (PCA) allowed to highlight natural grouping of samples. In addition, a linear discriminate analysis (LDA) model was also constructed to classify hempseeds according to harvesting time and cultivar.

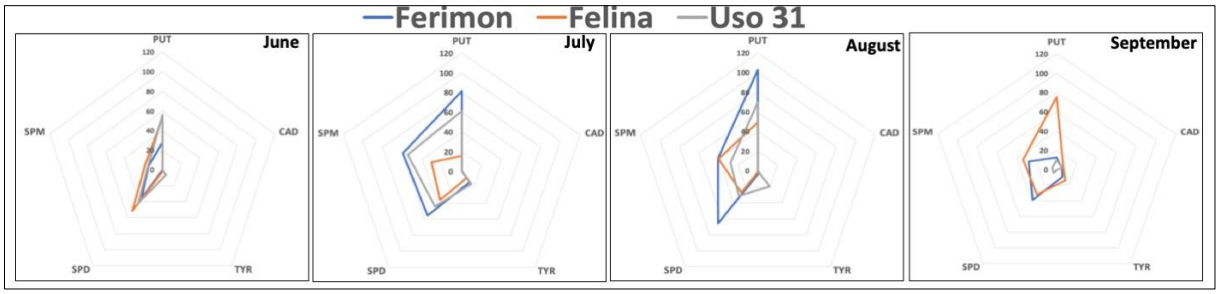

Fig.1. Average Biogenic Amine contents (mg/Kg) spider plots at different harvesting time.

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The functional properties, microstructure and amino acid composition of protein from safflower seed meal obtained by Subcritical Defatted Technology

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The objective of the present study was to investigate the influence of subcritical technology on the properties and microstructure of protein from safflower seed meal (SSM). The experiment of the functional properties of SSM protein indicated that the capacity of water absorption, fat absorption and emulsion stability of Subcritical butane extraction (SBE) were higher than that of Soxhlet extraction (SE) while there is no difference in foamability between two kinds of protein. The assay of the scanning electron microscopy (SEM) showed that the particles of SSM protein prepared by SBE were larger and protein molecules were more full and smoother with large pores around them compared to that of SE. The study of amino acid composition (AAC) testify showed that total essential amino acids of prorein prepared by SBE was significantly higher than of SE. In addition, the result of gas chromatography-mass spectrometry (GC-MS) showed that total amount of unsaturated fatty acids of SBE was higher than SE (79.97g/110g and 77.63g/100g, respectively).

Keywords: subcritical technology; safflower seed meal protein; functional properties; microstructure; amino acid analysis.

Fingerprint of sugar and organic acid by HPLC for authentication of peach juice

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Background Sugars and organic acids are the main nutrients and taste components in fruit juices, which show a lower susceptibility to changes as compared with other components such as pigments, antioxidants, and flavour compounds[1]. Therefore, characterization of the composition of sugar and/or organic acid in fruit juices is considered as a desirable approach for fruit juice authenticity as well as quality control[2,3]. Chromatographic fingerprint analysis is regarded as a rational approach for assessing the quality of raw materials and/or their processing products, which provides analytical signals related to the composition of foodstuffs in a non-selective way such as by collecting a spectrum or a chromatogram[4].

Objectives This work aims to assess the possibility of authentication and quality control of peach juice with HPLC fingerprint related to sugar and organic acid profile.

Methods Based on the method for high performance liquid chromatography (HPLC) analysis of sugars and organic acids in peach juice, the HPLC fingerprint of peach juice was developed by using the software of Similarity Evaluation System for Chromatographic Fingerprints of Traditional Chinese Medicine (Version 2.0), then similarity analysis, principal component analysis coupled to fingerprint were used to classification and discrimination the peach juices adulteration with apple juice, pear juice, and exogenous sugar/citric acid. The chromatograms of juices prepared from different peach varieties and harvest times with a total amount of 28 were extracted for development of fingerprint.

Results Sugar and organic acid standard fingerprints were established and the common fingerprint peaks were identified by using the chromatograms of 28 peach juice samples, and the similarities between fingerprints of reference and peach juices were above 0.960 for sugar and 0.948 for organic acid. This indicates that fingerprints of peach juices of different varieties and harvest times showed good consistency. Adulteration with apple juice, pear juice and exogenous sugar/citric acid led to decrease of similarity. The adulteration juice samples with addition of 40% of apple or pear juice could be discriminated from peach juices by using sugar fingerprint, while the limit was 60% by using organic acid fingerprint. When the peach juice was added into 30% of syrup, the adulteration samples could be discriminated by using sugar fingerprint. The adulteration juice samples with addition of 0.05% of citric acid could be detected by using organic acid fingerprint. All the adulteration samples could be discriminated from peach juices by using the HPLC fingerprints of sugars and organic acids coupled to principal component analysis except the sample with addition of 10% of syrup. In addition, this research also revealed the common profile of sugar and organic acid composition of peach juices prepared from different peach varieties and harvest times. Sucrose was the most abundant sugar in peach juice, accounting for 58.26–77.11% of the total sugar content. Quinic acid, malic acid and citric acid were the main organic acid in peach juice, which contributed to 18.48–57.87%, 23.71–60.43% and 8.29–29.25% of the total acid content.

Conclusion The peach juice adulteration with apple juice, pear juice, exogenous sugar (above 20%) and citric acid could be discriminated by HPLC fingerprint related to sugar and organic acid composition combined with chemometrics, which may be useful for the authentication and quality control of peach juice.

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The authenticity assessment of bilberry juice using GC×GC-TOFMS

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Bilberry is characterized by high nutritional value, therefore it is referred to as functional food. It has anticancer, anti-inflammatory and antibacterial properties. Bilberry juice can constitute an element of a daily diet with a positive effect on health and the human body. The healthiest type of fruit juice is directly squeezed juice. Due to high production costs, these beverages are often adulterated. Bilberry juice is sometimes falsified with mulberry juice because this addition is easily available and cheap. The addition of a different type of juice causes deterioration of juice quality, changes its organoleptic properties and nutrient content. It is important to assess the authenticity of fruit juices.

The purpose of the work was to characterize the volatile fraction of bilberry and mulberry juices, as well as to select key aroma compounds and to assign them descriptors of aroma and taste. In addition, the selection of potential discriminants indicating the adulteration of bilberry juice with mulberry juice is the main goal.

The research involved the use of two-dimensional gas chromatography coupled with mass spectrometry. It allows the identification and determination of volatile organic compounds with very low concentrations in samples with a complex matrix. In addition, the results of the volatile fraction analysis using the instrumental method were compared with the results of the sensory evaluation.

On the basis of the obtained results, potential discriminants of bilberry juices with mulberry juice, namely: 2,3-butanediol, 2,3-butanedione, acetoin, butyrolactone and ethyl lactate were selected. The distinguished chemicals have creamy, buttery, milky and fruity odour and flavour type. Presented solution can be treated as an alternative to other analytical techniques used for determining fruit juice adulteration.

Acknowledgments: Participation in the conference was financed within the project POWR.03.05.00-00-Z044/17 *Integrated Programme of Development of Gdańsk University of Technology*.

Assessment of the possibility of using waste from Kaffir lime fruit (*Citrus Hystrix*) as raw materials for industrial purposes based on the determination of the content of health-promoting compounds

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The issue of managing citrus wastes is an extremely important issue for environmental and economic reasons. Food processing plants are struggling with the problem of proper post-production waste treatment. There are many ways to use citrus waste, however, due to the increasing demand for fruit juices, related to the community's desire to live a healthy lifestyle, the amount of waste generated constantly increases. The Food and Agriculture Organization of the United Nations estimated that in 2013 the amount of industrial waste from the production of orange juice was 14.3 million tons [1]. Therefore, new solutions thanks to which it is possible to utilize citruses wastes are sought.

The problem of waste management is particularly important in the case of Kaffir lime, which is the most popular citrus in Southeast Asia. Leaves and outer parts of the peel are the only parts of fruit, which are consumed. They are used as spices and additions to many dishes. The juice is not consumed because of too tart flavor and in the case of Kaffir lime, 95% of the fruit is industrial waste.

During the research, it was shown that Kaffir lime peel and juice are a valuable source of health-promoting compounds and that these chemical compounds can be obtained on an industrial scale from the remains of Kaffir lime. Due to the high content of terpenes with health-promoting properties in citrus peel, Kaffir lime can also be an excellent raw material for acquiring essential oils used in the cosmetics or pharmaceutical industry.

Juice, pulp and Kaffir lime peel were the objects of investigation. Two-dimensional gas chromatography coupled with mass spectrometry and solid phase microextraction has been used to carry out determinations of selected terpenes in Kaffir lime samples. The developed of analytical methodology allows for the determination of fruit in samples without the need of distillation or solvent extraction.Terpinen-4-ol and citronellal appeared to be the most important constituents of *Citrus hystrix* with the highest concentrations in the peel (34.58±0.75 µg/g) and pulp (66.02±0.85 µg/g). The content of terpinen-4-ol in the Kaffir lime juice is 44.79±1.09 µg/g. In addition, polyphenols have also been determined in the Kaffir lime fruit samples showing the following content:23.16±2.18 mgGAE/g DW for juice, 22.14±1.5 mgGAE/g DW for pulp and 39.85±3.1 mgGAE/g DW for peel.

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The impact of different processing methods on the phenolic acid profile, antioxidant activity and ACE inhibition of white beetroot

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The plant variety and technological processes may significantly affect the content and composition of phenolic acids in vegetables and their products. In recent years, phenolic acids have attracted interest of scientists, due to their many potential benefits (antioxidant, antibacterial, antimicrobial, antimutagenic, anti-inflammatory, vasodilatory and anticarcinogenic activities) [1,2]. Furthermore, the novel variety of beetroot such as white beetroot could be a good source of these compounds.

The aim of this study was to investigate the impact of three different methods of treatment (boiling, baking and fermentation) on the phenolic acid composition, antioxidant capacity and ACE inhibitory activity of white beetroot. The 'Śnieżna Kula' white beetroot (*Beta vulgaris* L.) was used in the study. Roots were grown in the experimental fields of the TORSEED S.A. – Garden Seed and Nursery Stock Company in Toruń (Poland). Extracts from lyophilized samples were subjected to multistage extraction with a mixture containing water/methanol (20/80, v/v). Phenolic acids (free and those released from soluble esters and soluble glycosides) were isolated from the obtained extracts according to the method described by Wiczkowski et al. [3]. Then, the obtained extracts were analyzed using the micro-HPLC-QTRAP-MS/MS method. The antioxidant capacity (AC) was determined by four in vitro assays: PCL ACW, PCL ACL, FRAP and CUPRAC. In addition, inhibition of angiotensin-1 converting enzyme (ACE) by white beetroot's extracts were measurement.

By means of the micro-HPLC-QTRAP-MS/MS method nine phenolic acids were detected, including five derivatives of hydroxycinnamic acid and four derivatives of hydroxybenzoic acid. To the hydroxycinnamic acids group belong chlorogenic, sinapic, ferulic, caffeic and *p*-coumaric acids, while hydroxybenzoic acids were represented by vanillic, protocatechuic, syringic and 4-hydroxybenzoic acids. The dominant compounds in the white beetroot products were ferulic and *p*-coumaric acids, constituting 65% and 20% of the total phenolic acid content, respectively. The content of phenolic acids increased in the boiled white beetroots (~3%); however, baking and fermentation reduced the level of these compounds by approximately 6% and 11%, respectively. Among the white beetroot products the highest antioxidant activity values and ACE inhibition was observed for fermented roots. A significant positive correlation was measured between the results of all AC assays, and sinapic and caffeic acids content. The ACE inhibitory activity of white beetroot may be attributed to syringic and/or 4-hydroxybenzoic acids. In general, the results demonstrated that the applied treatments led to change concentrations of total and individual phenolic acids as well as antioxidant capacity and ACE inhibition.

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Comparison of fatty acid profile of eight meat-cuts in pigs commonly produced in France

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Fatty acid (FA) profiles of 8 meat-cuts used in processed meats are presented in table 1. Animals were slaughtered in 3 slaughterhouses, in autumn and spring to obtain as large a variation in FA contents as possible for pigs commonly produced in France. Each sample was a blending of meat-cuts from a female and a castrated male. The carcass weights ranged from 90 and 96 kg, the lean meat content from 59 and 63%.
Total fat and FA composition were determined (Rule et al,1997). Data were analysed using the GLM procedure (SAS v 9.4), differences between samples were assessed by the Tuckey test.

The fatty acid data (Table 1) indicated a dominance of C18:1n-9, C16:0, C18:0 and C18:2n-6 across all cuts of the carcass in agreement with other literature (Enser et al,1996, 2000). Saturated fatty acids (SFA) percentages were between 35.3 and 40.5%, nevertheless hypercholesterolemic FA C12:0 and C14:0 were very low 0.10% and 1,28 to 1.44% respectively. Mono unsaturated fatty acids (MUFA) represented almost the half of FA with a minimum of 45.3% for the backfat. The major polyunsaturated fatty acid (PUFA) C18:2n-6 derived entirely from the diet showed higher proportions in backfat (12.1 %) than in the loin (9.3 %), the second PUFA was 18:3n-3, but at lower level than 18:2n-6, from 0.58% in loin to 0.86% in backfat, these results are in accordance with Kouba et al (2003). Long chain n-3 and n-6 PUFA were present at low percentage. Total fat in cuts from ham, shoulder or loin were higher (6.55 to 8.84 g/100g) than in other studies in which no adhering subcutaneous or inter-muscular adipose tissue, have been examined. As the study aim was to examine muscle and fat tissues as normally used by meat processors, only rough dissection was performed.

Table 1. Total fat content (g/100g) and fatty acid composition (% fatty acid) of the 8 meat cuts (n=9)

	Backfat	**Rind**	**Jowl**	**Picnic**	**Ham**	**Shank**	**Shou.**[A]	**Loin**	**RMSE**[B]	**p-value**
Total fat	80.6^{a}	12.9^{c}	47.4^{b}	11.7^{c}	6.6^{d}	7.1^{d}	8.8^{cd}	6.7^{d}	3.033	***
SFA	**40.0^{ab}**	**35.3^{d}**	**38.4^{abc}**	**38.1^{abc}**	**37.7^{bcd}**	**37.0^{cd}**	**39.3^{abc}**	**40.5^{a}**	**1.747**	*******
C12:0	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.000	ns
C14:0	1.42^{ab}	1.39^{ab}	1.44^{a}	1.36^{ab}	1.28^{b}	1.28^{b}	1.34^{ab}	1.30^{ab}	0.102	**
C16:0	24.5^{a}	23.2^{b}	24.1^{ab}	23.7^{ab}	23.3^{ab}	23.0^{b}	24.2^{ab}	24.4^{a}	0.849	***
C18:0	13.1^{ab}	10.0^{c}	12.1^{b}	12.2^{b}	12.3^{b}	11.8^{b}	12.8^{ab}	13.9^{a}	0.929	***
MUFA	**45.3^{b}**	**50.5^{a}**	**48.8^{a}**	**49.0^{a}**	**49.0^{a}**	**50.3^{a}**	**47.6^{ab}**	**47.3^{ab}**	**2.173**	*******
C16:1n-7	2.33^{c}	3.27^{a}	2.89^{ab}	2.96^{ab}	2.89^{ab}	2.96^{ab}	2.92^{ab}	2.61^{bc}	0.282	***
C18:1 n-9	38.5^{b}	41.3^{a}	40.9^{ab}	40.4^{ab}	40.4^{ab}	41.3^{a}	39.0^{ab}	39.4^{ab}	1.788	**
C20:1n-9	0.79^{ab}	0.89^{a}	0.77^{b}	0.74^{b}	0.79^{ab}	0.81^{ab}	0.78^{b}	0.77^{b}	0.072	**
PUFA	**14.2**	**13.6**	**12.3**	**12.4**	**12.7**	**12.3**	**12.6**	**11.8**	**2.052**	**ns**
C18:2 n-6	12.1^{a}	11.2^{ab}	10.1^{ab}	9.9^{ab}	9.8^{ab}	9.4^{b}	9.9^{ab}	9.3^{b}	1.684	*
C18:3n-3	0.86	0.81	0.79	0.66	0.60	0.62	0.62	0.58	0.190	**
C20:4n-6	0.20^{c}	0.46^{bc}	0.30^{c}	0.86^{ab}	1.09^{a}	1.06^{a}	0.90^{a}	0.89^{a}	0.286	***
C20:5n-3	0.00	0.01	0.00	0.01	0.02	0.01	0.00	0.01	0.028	ns
C22:5n-3	0.10^{b}	0.10^{b}	0.10^{b}	0.12^{ab}	0.18^{a}	0.14^{ab}	0.18^{a}	0.16^{ab}	0.049	***
C22:6n-3	0.02	0.00	0.01	0.00	0.02	0.04	0.01	0.02	0.037	ns

Shou.[A] shoulder upper half; **RMSE**[B] root mean square error; a-d Within a row, means lacking common superscript letters differ (P < 0.05) ;. ns= P N 0.1; * = P ≤ 0.05; **= P ≤ 0.01; *** = P ≤ 0.001.

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Effects of the Degree of Ripeness on the Phenolic and Carotenoid Profiles in Different Tissues of *Citrus* Fruits from Southern Italy

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Citrus are fruit crops that are consumed worldwide and have a great importance in the agricultural sector of Southern Italy. The region of Sicily is one of the largest producer of citrus fruits in Europe, and about 60% of the fruits produced in Italy are produced in this region.[1] Citrus fruits are popular amongst consumers due to their pleasant organoleptic characteristics and the high content of bioactive compounds, e.g., carotenoids and phenolic compounds. To date, a large number of phytochemicals have been identified in juices from ripe fruits, however, few investigations have researched the changes in phytochemicals occurring in the different tissues, e.g., albedo, flavedo and juice vesicles, during fruit development. In this study, the effects of the ripening stage on the different tissues of four citrus cultivars grown in Sicily, Southern Italy, were investigated.

Fruits from pummelo (*C. grandis* L. Osbeck) cv 'Chandler', lemon (*C. limon* L. Burm. f) cv. 'Akragas', and sweet orange (*C. sinensis* L. Osbeck) cvs 'Tarocco 57-1E-1' and 'Washington navel', were grown in the experimental fields of Acireale (37°36′31″ N, 15°09′56″ E) and Palazzelli (37°20'22" N, 14°53'31'' E), Sicily, Southern Italy, and harvested every five weeks from September 2018 to January 2019. In total, fruits were collected at four ripening stages, from immature to full maturity. Fruits were washed with distilled water, and manually sectioned in albedo, flavedo and juice vesicles. Then, the citrus tissues were stored at -80°C, freeze-dried, milled, and kept under vacuum at RT.
Phenolic compounds were extracted and analysed by UHPLC-MS/MS as described by Ferguson *et* al (2018).[2] Carotenoids were extracted and analysed by HPLC-DAD as described by Multari *et* al (2018).[3]

In all the selected fruits, albedo and flavedo had higher cumulative phenolic contents than the juice vesicles, at all the ripening stages. Hesperidin resulted the main phenolic compound in the tissues of *C. limon* and *C. sinensis* cvs Tarocco and Washington, whereas *C. grandis* was the richest in narirutin, e.g., 4172 ± 240 mg kg^{-1} dw (unripe fruit). Overall, cumulative phenolic acids reduced during ripening, whereas elevated levels of flavonoids were detected at full maturity. Caffeic, *p*-hydroxybenzoic and ferulic acids were major compounds in the juice vesicles of unripe *C. grandis*, whereas apigenin-7-*O*-glucoside was the predominant compound in the flavedo from full mature *C. limon* (43.1 ± 6.11 mg kg^{-1} dw).
The carotenoid composition of the selected fruits resulted diverse. Across the samples, the flavedos were the tissues with the highest carotenoid content, and distinguished from albedos and juice vesicles due to great levels of lutein, *β*-cryptoxanthin, and (*all-E*)-violaxanthin. *(all-E*)-Violaxanthin increased significantly ($p < 0.05$) in the flavedo of *C. limon* during ripening. Juice vesicles showed carotenoid contents that increased constantly during ripening. *C. sinensis* cv. 'Washington navel' had juice vesicles with the highest content of cumulative carotenoids.

Results from the present investigation showed that the levels of phenolic compounds and carotenoids of citrus fruits from Sicily changed significantly over the ripening process. Overall, the tissues flavedo and albedo resulted valuable sources of phytochemicals. This information is useful for a comprehensive utilization of all the citrus tissues by the food industry.

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A novel approach for food authentication using trace element labelling and laser ablation ICP-MS

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Food labelling with trace elements constitutes a promising alternative to food authentication by means of chemical fingerprinting. In this approach, a distinctive alteration to the natural trace element distribution is introduced via spiked feed for animals or spiked soil or water for plants, which can be detected in the food product using suitable analytical techniques. Therefore, products of a certain origin can be labelled in order to discriminate them from unlabelled products of other origin. Trace element labelling is more or less universally applicable and requires only very small amounts of spike elements if the natural abundance of the elements chosen is sufficiently low, ensuring safety for the customer.[1,2]

In this contribution we describe detection of rare earth element (REE) labels in food samples, such as eggs, chicken and lamb meat, and goat milk. Due to the generally low REE levels in animal tissues, their quantitation can be quite challenging. A combination of acid digestion and inductively coupled plasma mass spectrometry (ICP-MS) is most commonly applied. However, the large amounts of matrix elements such as Ca contained in samples such as egg shells or bones may impair ICP-MS measurement. Typically, these issues are addressed by sample dilution, at the cost of lower sensitivity. In comparison, laser ablation ICP-MS (LA-ICP-MS) is less prone to some matrix effects, and measurements can be performed with limited or even without sample preparation. In samples with high inorganic content, such as egg shells or bones, LA-ICP-MS allows direct detection of REE labels. Samples with higher water and/or organic content, such as milk or meat, are prepared by dry ashing and pressing pellets. Thus a considerable enrichment of inorganic constituents, and therefore also REE, is achieved which compensates for the lower sensitivity of LA-ICP-MS compared to solution based ICP-MS.

Results show that both methods are capable of detecting REE labels at ultra-trace levels in food products, allowing distinction of labelled from unlabelled products. This novel approach could potentially be applied to specifically label foodstuffs produced in certain regions or under certain conditions, in order to ensure food authenticity.

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Volatile Compounds of Different Fresh Wet Noodles Cultivars Evaluated by Headspace Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry

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Asian noodle has a history of thousands of years. Nowadays, in order to enrich the varieties of noodles, wheat noodles have been fortified with various ingredients. The three major components of flavor are taste, texture and aroma. This paper will focus primarily on noodles produced from potato flour and the different flavor between potato noodles and wheat noodles.
Noodles were produced with different materials (Table 1). The headspace solid phase microextraction (HS-SPME) was conducted using a SPME fiber. The 2 cm fiber had a 50/30 μm divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/Carboxen/PDMS) coating bonded to a fused silica core (Agilent Inc., USA). Seven grams of the sample were placed in a 20-mL capped vial with 1 μL of 0.816 μg/mL 2,4-dimethyl-3-heptanone hexane solution as the internal standard. The GC-MS analysis was performed using an Agilent 7890B gas chromatograph equipped with a DB-WAX capillary column, well-suited to analyzing complex volatile compounds, coupled to an Agilent 5977A MSD (Agilent, USA).
Different noodles were analyzed using SPME-GC-MS technique, and the conclusions are as follows: (1) Volatiles of noodles composed of mainly of aldehydes, alcohols, ketones, esters and organic acids. The species of the total volatile compounds of cooked noodles was higher than the raw noodles. (2) The species of the total volatile compounds of the samples potato/wheat flour noodles, containing mainly aldehydes compounds, was higher than wheat noodles. (3) With the storage time increased, organic acids increased significantly at 30 h for the potato/wheat flour noodles.

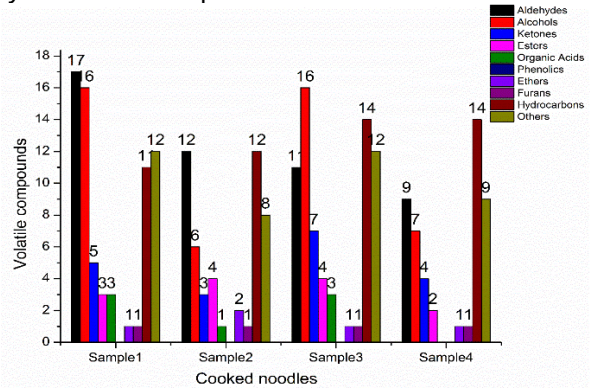

Fig.1 Volatile compounds identified in potato/wheat flour and wheat flour noodles samples

Table 1 Gluten and moisture contents of the noodles

	Potato flour added (g)	Wheat flour added (g)	Distilled water added (g)	Salt added (g)	Alkali added (g)
Sample 1	40	60	56	0	0
Sample 2	40	60	56	0.2	0
Sample 3	40	60	56	0.2	0.1
Sample 4	0	100	33	0	0

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From octanol-water to oil-water systems: a comparison of the partition constant values of antioxidants

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Partition constant values of bioactive compounds between octanol and water, P_W^{OCT}, are extensively used in predictive environmental and pharmaceutical studies (1) and are important for a rational description of their partitioning behavior in multiphasic systems. The partition constant between edible oils (e.g., olive) and water, P_W^O, correlate pretty well with their partitioning into rats and human tissues. In some instances, they are even superior for the prediction of lipid tissue plasma partition coefficients over the widely used octanol−water partition constant, P_W^{OCT} (2). Knowledge on how antioxidants (AOs) distribute in different systems and the effects of hydrophobicity of AOs on their distribution in binary edible oil−water systems is important in relevant economic areas such as medicine, pharmacy, and food technology.

Data on distribution of antioxidants in edible oils, and particularly in olive, soybean and corn oils, are scarce and much more sparse than partitioning data in octanol−water systems. Here, we evaluated the partition of series of homologous antioxidants (AOs) of different hydrophobicity derived from potent, natural AOs in edible oil−water binary systems. For the purpose, we grafted gallic (GA), caffeic (CA), and protochateuic (PT) acids and hydroxytyrosol (HT) with alkyl chains of increasing hydrophobicities (1,3) so that four series of homologous AOs bearing the same reactive group but of different alkyl chain length were prepared.

Results show that the variations of the partition coefficients with the number of C atoms in the alkyl chain were linear and parallel to those obtained in octanol−water, indicating a similar contribution of the $-CH_2$ groups to the total hydrophobicity of the molecule in both solvents. The intercept values, which reflect the contribution of the nonalkyl portion of the molecule, are different for all AOs as a consequence of the different groups in the chemical structures of the AOs. When AOs contain ionizable groups (phenolic and ascorbic acids), the values of the partition constants depend on the actual value of the acidity, decreasing upon decreasing acidity following a sigmoidal-like curve. Results demonstrate that, in general, P_W^O values cannot be predicted from P_W^{OCT} values and should be evaluated for each series of AOs employed.

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Study of element contents in bivalve molluscs: health benefit and risk

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Bivalve molluscs, which include mussels, oysters and clams, have high nutritional value. They are regarded as a good source for proteins, lipids, carbohydrates and minerals [1]. On the other hand, seafood may also contain harmful contaminants and other undesirable substances such as mercury and persistent halogenated compounds, which has resulted in a number of risk-benefit assessments during the last decade [2].

Four species of bivalve molluscs *Ruditapes philippinarum (*Manila clam, MC), *Yesso scallop* (YS), *Tegillarca granosa* (TG) and *Anadara broughtonii* (AB) were bought in two fish markets in Incheon, Korea, in order to determine content of As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Zn, and Fe and consequently, to estimate the health hazards associated to dietary intake. The samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after closed-vessel microwave digestion. The analytical accuracy of the method was evaluated by using the SRM (TORT-2, lobster hepatopancreas). Application of principal component analysis (PCA) and hierarchical cluster analysis (HCA) showed a tendency to form three groups between samples belonging to different genus of samples.

European food safety authority (EFSA) has established recommended daily intake (RDI) values for Cu, Fe, Mn and Zn of 3, 14, 5 and 10 mg/day, respectively. We calculated the RDI for daily consumption in milligrams per 300 g of sample. Our results showed that these species could serve as a good dietary source of essential elements, especially Fe, Mn and Zn. However, all species showed As content higher than the maximum tolerable limit specified by EFSA. Seafood is the major contributor to As in the diet though As in seafood mostly occurs as organic As species [3]. In addition, content of Mn in Yesso scallop is few times higher than in other species.

Table 1. Results found in bivalve molluscs species (mg/300 g, w.m.)

Elements	MC	AB	TG	YS
As	0.78 ± 0.09	0.42 ± 0.23	0.45 ± 0.15	0.31 ± 0.08
Cd	0.03 ± 0.01	0.15 ± 0.14	0.26 ± 0.16	0.34 ± 0.27
Co	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
Cr	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Cu	0.23 ± 0.04	0.32 ± 0.19	0.36 ± 0.18	0.31 ± 0.27
Hg	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.01
Mn	0.40 ± 0.15	1.08 ± 0.70	1.12 ± 0.52	17.8 ± 17.5
Ni	0.17 ± 0.05	0.02 ± 0.01	0.04 ± 0.01	0.10 ± 0.11
Pb	0.01 ± 0.02	0.03 ± 0.02	0.04 ± 0.04	0.02 ± 0.01
Se	0.16 ± 0.03	0.10 ± 0.04	0.16 ± 0.04	0.12 ± 0.03
Zn	2.51 ± 0.29	3.53 ± 0.92	3.74 ± 0.79	9.15 ± 6.19
Fe	10.5 ± 6.6	16.7 ± 8.7	20.6 ± 6.9	4.4 ± 2.6

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Analysis of Volatile compounds in fresh sturgeon with different preservation methods using electronic nose and GC/MS

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Contamination of microorganisms caused rapidly quality deterioration of fresh sturgeon meat, which results in shortening the shelf-life and health risk. In this paper, two preservation treatments based on microbial control were taken. During the chilling storage, the sensory analysis and volatile compounds (VOCs) evaluation were performed by electronic nose and SPME-GC/MS. Results showed that washing with acidic oxidized electrolyzed water and the addition of ε-polylysine had influences on sensory VOCs of the fresh sturgeon by inhibiting spoilage of microbes or introducing chemical agents like free 2chlorine and reactive oxygen species. Analysis of GC/MS detected more than 40 kinds of VOCs in the fresh sturgeon during chilling storage, mainly aldehydes and ketones. Relative content of heptanal, nonanal, and acetophenone increased linearly with the storage time in all groups, but that of hexanal and octanal decreased. It indicated the work found the potential bio-markers, acting as indicators for evaluating quality of sturgeon products rapidly.

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Fatty acids composition of the most common bivalves in Korean diet

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Consumption of bivalve molluscs, such as oysters, mussels, clams and scallops, makes a significant part of the daily Korean diet. Bivalves provide high quality proteins with all the dietary-essential amino acids, lipids, vitamins, minerals and other bioactive nutrients, which offer a variety of health benefits to the consumer [1]. This food contains less than 5 percent of total fat, so it is considered a low-fat food. Beside the amount of total fat, the proportions of saturated, monounsaturated and polyunsaturated fatty acids (FA) (S, M and P, respectively), as well as ratio of n-3 (ω-3) and n-6 (ω-6) P in food are very important for the health diet [2].

Fourteen species of bivalves *Anadara broughtonii* (AB), Ruditapes philippinarum (Manila clam (RP)), Tegillarca granosa (TG), Pecten yessoensis (Yesso scallop (YS), Argopecten spp. (small scallop (SS), Chlamys farreri farreri (CF), Cyclina sinensis (CS), Leukoma jedoensis (LJ), Mytilus californianus (MCa) Mytilus galloprovancialis (MG), Maretrix lusoria (ML), Mactra quadrangularis (MQ), Sinovacula constricta (SC) and Crassostrea gigas (Pacific oyster (PC)) were bought in two fish markets in Incheon, Korea, in order to determine FA composition using GC/EI-MS of fatty acid methyl esters (FAME). The FAME were identified by comparing their retention times with those of the FAME standards or by comparing their mass spectra with those stored in the NIST Mass Spectral Library.

In the bivalve samples, 43 different FA were identified, of which 10 were S, 12 M and 13 P, other FA were 7 methyl-FA and 1 hydroxyl-FA. The P/S ratio and ω-6/ω-3 P ratio are the most significant markers of lipid composition in a healthy diet and both should be close to 1 [3]. Among analysed species, only YS and SS have P/S ratio close to 1 (1,20 and 1.16, respectively), while other species have value between 0.07 and 0.73. The obtained values for ω-6/ω-3 P ratio were from 0.008 to 0.55, which indicates that bivalve molluscs are the valuable source of ω-3 P (EPA and DHA). These ω-3 P play important roles in growth, development, and maintenance of health.

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Amino acid content of Aragonez grapes: the influence of soil fertilization

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Grape soil fertilization has been used to enhance the quality of grapes and therefore wines. Vineyard fertilization practices aim to ameliorate the supply of available soil nutrients to the levels required for optimum grapevine growth and yield. Most soils will contain adequate amounts of micronutrients. However, nitrogen, phosphorus and potassium (principal macro-nutrients) as well as magnesium, calcium and Sulphur (secondary macro-nutrients) are the ones that usually can limit grape production. Magnesium tends to be deficient in vineyards if the soil pH is too low, and Alentejo soils usually have low pH. Magnesium is required as a component of chlorophyll molecules and for metabolic processes and influences fruit formation and berry ripening.

The aim of this work was to understand the influence of several nutrients' application on soil vineyards on the amino acid content of the must from Aragonez grapes. The experiment was conducted in a randomized block design, with three replications, in a split-plot arrangement. Two different doses of Mg were applied (D1 and D2). For each one there was six different treatments: 1) with N, P, Ca, S, K; 2) with P, Ca, S, K ; 3 with N, Ca, S, K; 4) with N, P, S, K; 5) with N, P, Ca, K; 6) with N, P, Ca, S. A control plot with N, P, Ca, S, K but without Mg addition was also considered, in a total of 39 plots. Grapes were picked up at harvest ripening and must was kept at -80°C until analysis. The amino acid content of the must was quantified using a HPLC Waters Alliance System 2695-series Separation Module equipped with Alliance Series Column Heater and the detection was carried out using a photodiode array detector (2998 PDA Detector) (Waters, USA). The column used was an ACE HPLC column (5 C18-HL) particle size 5 μm (250 mm x 4.6 mm). Prior to injection, samples were derivatized. Results shows that some differences among the amino acid content of the grape must samples can be observed.

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Determination of Soluble and Insoluble Dietary Fiber Content of Marine Macroalgae and Microalgae

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Diet composition, daily dietary intake and acute dietary changes have a big impact on modulating the microbial composition of the gut [1]. In fact, nowadays scientists recognize that diet is a key environmental factor for modulation of gastrointestinal microbiota composition and metabolic function and that the consumption of specific dietary ingredients such as fibers is an excellent form to benefit human gut microbiota and overall health since it is correlated with metabolic, immunologic, and protective functions in the human organism [1-4]. Seaweeds and microalgae are excellent sources of potential prebiotic fibres such as fucoidans, alginates, carrageenans and exopolysaccharides that are not digested, and are then selectively fermented by beneficial colonic microbiota [5]. In this work the soluble and insoluble fiber contents of five macroalgae (*Palmaria palmata, Gracilaria gracilis, Porphyra spp, Ulva rigida and Fucus vesiculosus)* obtained from land-based cultivation systems, under the Integrated Multi-Trophic Aquaculture (IMTA) sustainable concept, and three microalgae *(Chlorella vulgaris,* organic certified *Chlorella vulgaris and Tetraselmis chuii)* all cultivated in fully controlled closed systems, were estimated following the acid detergent method developed by Van Soest *et al* [6]. Macroalgae revealed to be an excellent source of total fibers with values ranging from 4.7±0.1 (*P. palmata*) to 37.0±0.4 %(w/w) (*F. vesiculosus*) – insoluble fibers registered values between 1.33±0.05 (Porphyra spp.) and 22±2 %(w/w) (*F. vesiculosus)* and soluble fibers between 1.7±0.2 (*P. Palmata*) and 20.4±0.8 %(w/w) (*Porphyra spp.*). In comparison with macroalgae, microalgae present lower fiber contents with values ranging from 0.84±0.04 (*Chlorella vulgaris*) to 2.42±0.06 %(w/w) (*Tetraselmis chuii*) – insoluble fibers registered values ranging from 0.39±0.06 (*Chlorella vulgaris*) to 1.3±0.2 %(w/w) (Organic *Chlorella vulgaris*) and soluble fibers between 0.4±0.2 (Organic *Chlorella vulgaris*) and 1.81±0.07 %(w/w) (*Tetraselmis chuii*). Interestingly the organic *Chlorella vulgaris* presented almost double the content of total dietary fiber compared to the conventional counterpart (1.63±0.21% and 0.84±0.04 %(w/w), respectively). These marine resources, and particularly macroalgae, present an excellent source of soluble and insoluble fibers that could act as prebiotics modulating positively human gut microbiota.

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Chemical composition and antimycoplasma activity of a brown propolis from southern Brazil

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Propolis composition depends on the resin plant source. Propolis types are characterized by the corresponding chemical composition and resin plant source[1]. Several types of Brazilian propolis have been characterized, such as the green type, derived from *Baccharis dracunculifolia*, and the red propolis, derived from *Dalbergia* ecastaphyllum[2].

Extracts of a sample of brown propolis produced in the district of Itapará (Southern Brazil) were obtained with solvents with increasing polarity.

The extracts were analyzed by RPHPLC-DAD-ESI-MS/MS and evaluated toward activity against *Mycoplasma bovis*, *M. gallisepticum*, *M. genitalium*, *M. hominis*, *M. hyorinis*, *M. penetrans* and *M. pneumonieae*[3,4].

Typical components of green propolis (e.g. prenylated phenylpropanoids, caffeoyl and feruloyl quinic acids) were characterized in the analyzed propolis, in addition to several flavonols (e.g. quercetin glycosides and kaempferide).

Less polar extracts showed higher anti-mycoplasma activity (MIC value commonly 3.9 µg/mL) than alcoholic and aqueous extracts (MIC value often 7.8-250 µg/mL). The results indicate that Itapará propolis is a promising source for the development of therapeutic drugs. Comparison of the results with literature data suggests higher activity of green propolis against mycoplasma than against cell wall-bearing bacteria.

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Effect of water stress on glucosinolate content and their breakdown in pak choi (*Brassica rapa* L. ssp. *chinensis*)

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Brassica species characteristically contain glucosinolates (GLS), which are sulfur-containing secondary plant metabolites with health-promoting effects in humans. When plant tissues are demaged, GLS are hydrolysed by the enzyme myrosinase, resulting in several degradation products, including isothiocyanates (ITC), nitriles (CN) and epithionitriles (EPT) [1]. Particularly ITC have chemopreventive effects and can protect cells against certain cancers [2]. Therefore it seems desirable to have *Brassica* vegetables rich in the formation of ITC. However, many *Brassica* vegetables rather release CN and EPT upon GLS hydrolysis and knowledge on abiotic factors affecting the hydrolysis outcome is scarce.

Since plants are often exposed to biotic and abiotic stresses, their traits vary as a result of responses to these. Drought is a major environmental stress that is expected to reduce some vegetative growth parameters with the subsequent increase of secondary metabolites at the expense of primary metabolism [3]. Previous studies indicated that water stress may modify GLS composition in most of the cruciferous vegetables [4, 5], but it is unclear whether the GLS breakdown is also affected.

In this study we investigated how different levels of water stress affect the formation as well as the hydrolysis of GLS in pak choi (*Brassica rapa* L. ssp. *chinensis*) cv. Black behi. Plants were grown in a climate chamber under controlled conditions. After 4 weeks the pots were watered to saturation. Then, plants were divided into 6 groups with different watering regimes (control, overwatering, 80%, 60%, 50% and 40% of the average daily water need) and harvested after 1 week. GLS contents were measured from lyophilized plant material using UHPLC-DAD. Measurement of GLS breakdown products was carried out using GC-MS from freshly harvested material after homogenization.

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Measurement of theobromine content for determining cocoa solids content in chocolate products-Authenticity confirmation

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The National Regulation in Serbia defines the requirements for the quality of chocolate products, amongst others, the minimum quantity of fat-free cocoa solids in them. The amounts of fat-free cocoa solids are, in SP Laboratorija, calculated by multiplying the spectrophotometrically determined content of theobromine with factor for conversion of theobromine into fat-free cocoa solids, which is determined based on typical values for the content of theobromine, moisture and fat in raw materials (cocoa mass or cocoa powder).

By analyzing the theobromine content of the raw materials, in the SP Laboratorija, over the past three years, the obtained results ranged from 0.84% -1.31% for cocoa mass and 0.91% -2.30% for cocoa powder. When determining the content of fat-free cocoa solids of chocolate products, SP Laboratorija usually does not have information on the quality of raw materials used, so the application of the general conversion factor for the calculation is unavoidable. The results of the analyzes performed in the period 2016-2018, indicate that more than 95% of analyzed samples of chocolate and milk chocolate from the territory of Serbia met the requirements of the National Regulation, regarding the content of fat-free cocoa solids. The unsatisfactory result of the content of fat-free cocoa solids in chocolate products can be due to an insufficient quantity of cocoa mass / cocoa powder in the final product, or their poor quality.

The lack of a limit for the minimum amount of theobromine in raw materials for production of chocolate products leads to the fact that raw materials with a significantly lower content of theobromine than usual can be found on the market. This can mislead a producer who has used a required quantity of raw materials (fat-free cocoa solids) in his product, but this quantity can't be verified analytically during the control, which leads to doubts about the authenticity of the product.

Regular analysis of the contents of theobromine in raw materials and the definition of the limits in which they should be, could influence the improvement of the quality of chocolate products by using less quantity of cocoa mass or cocoa powder of the appropriate quality in the production, or use of raw materials with low theobromine content, manufacturers can make ungrounded financial profit and mislead consumers in terms of the quality of the products they use.

Linking aroma attributes to volatile components of black ripe olives

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Black ripe olives (or Californian-style black olives) are one of the most important types of table olives commercialized worldwide, accounting for 40-50% of all table olives exported from Spain, the world's main producer and exporter of table olives [1] (ASEMESA, 2019). Although optimal processing conditions and microbial spoilage have been extensively studied in black ripe olives, literature concerning volatile composition and/or sensory characteristics is almost nonexistent for this product. Clear differences among the volatile compositions of black ripe olives produced in different countries have been found [2]. In addition, olive cultivar, grown area, and storage period have been recently demonstrated to affect the sensory profiles of black ripe olives [3]. However, no study relating the volatile components to sensory attributes of black ripe olives has been carried out to date.

In the present study, the aroma profile and volatile composition of a number of samples of black ripe olives from Manzanilla and Hojiblanca cultivars were analyzed with the aim to characterize this type of table olive. The aroma of samples was analyzed by a sensory panel using quantitative descriptive analysis (QDA), whereas the volatiles were analyzed by headspace solid-phase microextraction (HS-SPME) and gas chromatography coupled to mass spectrometry (GC-MS). Eleven aroma descriptors (briny, sautéed mushroom, earthy/soil-like, oak barrel, nutty, artificial fruity/floral, natural fruity/floral, vinegary, alcohol, fishy/ocean-like, and cheesy), which were previously used for the evaluation of the sensory profile of black ripe olives [4], were assessed by the panel.

The aroma profile between cultivars was found highly similar. Significant differences ($p<0.05$) in the aroma intensities of the descriptors were found only for the "briny" descriptor. A total of 74 volatile compounds belonging to different chemical classes were identified and semi-quantified, of which 17 contributed to the discrimination between Manzanilla and Hojiblanca black ripe olives. Partial least squares (PLS) regression was applied to establish correlations among the sensory attributes and the volatile compounds. A reliable PLS model was only obtained for "nutty" descriptor using 17 selected volatiles. Pyridine and two pyridine derivatives (3-ethyl-4-methylpyridine and 3-ethylpyridine), which showed a high correlation between them, along with 4-ethylbenzaldehyde and benzyl alcohol were found to be significantly correlated with this descriptor.

In summary, the data obtained have shown that with the analytical procedures used and chemometric techniques such as PLS regression, it has been possible to identify the compounds that could potentially contribute to the "nutty" attribute in black ripe olives. The PLS model developed in the present study could be used to predict the intensity of this attribute as a function of SPME-GC-MS data.

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Comparison on Quality Parameters in Soft Cheeses

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The present study aimed to determine the difference in the protein content, amino acid, and biogenic amines composition in soft cheeses produced from cow, goat and buffalo milk at the small scale in Kosovo. All cheese was made by self-fermentation method and were provided by farmers/households. Protein content of buffalo milk cheese was 11%, followed by 18% for cow cheese and 22% for goat cheese. No significant differences ($P \geq 0.05$) on the total amount of amino acids of cheese samples were found. However, there were significant differences between the amino acid compositions of soft cheese produced from buffalo milk compare to the cheese produced from cow and goat milk. Acidic amino acids occurred in higher amount in the Cow Soft Cheese (CSCH) and Goat Soft Cheese (GSCH) compare to Buffalo Soft Cheese (BSCH), while the content of basic amino acids was higher in BSCH compare to CSCH and GSCH. The main amino acids were glutamic acid (CSCH: 25.02%; GSCH: 25.04%; BSCH: 26.86%), proline (CSCH: 16.18%; GSCH: 15.84%; BSCH: 13.39%), leucine (CSCH: 8.79%; GSCH: 9.44%; BSCH: 9.8%), and aspartic acid (CSCH: 6.19%; GSCH: 5.73%; BSCH: 6.25%) representing 56% of the total amino acid content. Highest total content of essential amino acids were found in the goat soft cheese (43%), followed by buffalo soft cheese (41%) and the lowest content was found in cow soft cheese (40%). Among essential amino acids, the main representatives were Leu, Phe and Lys, while methionine, histidine, and isoleucine were found only in small quantities. Regarding free amino acid composition of cheese samples, gamma-amino butyric acid (CSCH: 22.2%; GSCH: 19%; BSCH: 21.9%), Pro (CSCH: 7.8%; GSCH: 6.7%;BSCH: 9.9%), Glu (CSCH: 2.3%; GSCH: 9.9%; BSCH: 13.9%), Asp (CSCH: 4.1%; GSCH: 2.9%; BSCH: 6.9%), Val (CSCH: 4.0%; GSCH: 4.6%; BSCH: 2.1 %), Leu (CSCH: 2.1%; GSCH: 16%;BSCH: 2.6 %), Phe (CSCH: 2.8%; GSCH: 5.4%; BSCH: 1.9 %), and Lys (CSCH: 11.8%; GSCH: 2.3%;BSCH: 11.4%) were the major components, representing 58% of the total free amino acid content in the cow soft cheese; 68% in the goat soft cheese and 70% in the buffalo soft cheese. Results for biogenic amines showed that buffalo soft cheese and cow soft cheese are free of biogenic amines, while in goat soft cheese histamine (468 µg/g), tyramine (544 µg/g), putrescine (78 µg/g) and cadaverine (102 µg/g) were detected.

Key words: Amino acid, Biogenic amines, Buffalo milk, Cow milk, Goat milk, Protein, Soft cheese.

Functional analysis of an extracellular fructanase in *Lactobacillus crispatus*

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Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder. It affects 11% of the adult population in the developed countries and more in developing countries. Whole grain rye bread has been recommended as a good source of dietary fiber but its consumption may trigger IBS symptoms due to the content of fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) [1]. A low FODMAP diet allows IBS patients to manage their symptoms but also reduces the intake of dietary fiber [2]. The application of specific FODMAP-degrading sourdough to wholegrain bread produces a low-FODMAP bread without compromising the content of slowly fermented dietary fiber. *Lactobacillus crispatus* FUA3459 was isolated from a zero-FODMAP rye sourdough and carries a putative extracellular fructanase (FruA) which may be responsible for fructans degradation in rye sourdough. FruA or homologues, however, have not been characterized in lactobacilli [3].

This study aimed to characterize FruA in *Lactobacillus crispatus* FUA3459. Expression and enzyme activity of FruA were monitored under multiple substrates and during rye sourdough fermentation. The enzyme carries an N-terminal signal peptide and a C-terminal surface protein domain, which indicates that it is exported from the cell and attached to the cell wall. Accordingly, the cell wall fraction had a much higher fructanase activity than the cytoplasmic fraction or the culture supernatant. Expression of *fruA* was observed in media containing sucrose, fructose, glucose, or FOS as sole carbohydrate sources. FOS and inulin hydrolysis of FruA were not subjected to carbon catabolite repression by glucose. Consistently, the gene expression and enzyme activity of FruA were comparable during sourdough fermentation with half-cut whole rye grain or rye flour. Fructans in rye grain were degraded during the first 12h fermentation. The identification of FruA in *Lactobacillus crispatus* 3459 promote the utilization of this strain in low-FODMAP fermenting food production and may also as a probiotic that hydrolyze larger prebiotics to provide accessible products for other microorganisms in the intestine.

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Bioactive and nutritional characterization of stinging nettle (*Urtica dioica* L.) harvested in Portugal

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Stinging nettle (*Urtica dioica* L.) is an edible wild plant known since ancient times for its dietary and therapeutic benefits. Despite being frequently perceived as being a weed, its use as human food has a long tradition since it was part of the ancient Greek and Roman cuisines and latter it was used as a traditional food by the native American Indians [1]. In Europe, stinging nettle has been consumed as food mainly in periods of famine and scarcity, such as wars. The leaves of this plant can be used in the confection of soups and other dishes such as omelettes, risottos, puree, tarts and consumed as cooked vegetable [2]. Although in the last decades the use of stinging nettle in gastronomy has fallen into disuse, as happen with other wild edible plants, thus it is still traditionally consumed in several regions of the world, such as the Mediterranean region, as part of a cultural and gastronomic heritage. Therefore, this study aimed to perform the nutritional, chemical and bioactive characterization of the leaves of different samples of stinging nettles harvested in Portugal.

Fresh plant specimens were collected in the wild in the beginning of March 2017, from two different regions of Portugal, Viseu (40° 39′ 39″ N, 7° 54′ 34″ E) and Vila Real (41° 17′ 45″ N, 7° 44′ 46″). Another sample was collected from the same place in the region of Viseu, three months latter, in June 2017. The samples were evaluated regarding their nutritional composition including moisture, fat, proteins and ash, according to AOAC official procedures, and carbohydrates were determined by difference. Fatty acids were determined by gas chromatography coupled to a flame ionization detector (GC-FID) and phenolic compounds by High Performance Liquid Chromatography coupled to a diode-array and mass spectrometry detector using the electrospray ionization interface (HPLC-DAD-ESI/MSn). The antioxidant activity was evaluated by means of three different *in vitro* assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power and inhibition of β-carotene bleaching. The antimicrobial susceptibility assay was performed using the Kirby-Bauer disc diffusion method against 4 Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*) and 4 Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*).

The leaves of stinging nettle samples presented a high percentage of moisture (78.5-83.7 g/100 g fresh leaves) with carbohydrates being the major macronutrient (47.5-50.0 g/100 g, dry basis), while fat was present in very low amounts (3.3-4.0 g/100g, dry basis). A total of 21 fatty acids were identified in the lipid fraction, with α-linolenic acid being the predominant one (41.9-51.3%). The qualitative profile among the 3 samples was identical, although quantitative differences were observed. Regarding phenolic compounds' composition, a total of 16 compounds, including phenolic acids and flavonoids, were identified and quantified, with only 5 being present simultaneously in the 3 analyzed samples (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, caffeic acid, isorhamnetin-3-O-rutinoside and quercetin-3-*O*-rutinoside). Although the sample collected in June in Viseu region was the one with lower content of phenolic compounds, it presented a similar antioxidant activity to the sample from Vila Real, which had the highest content of phenolic compounds. In general, the extracts showed a low activity towards the tested bacteria, with the exception for *Pseudomonas aeruginosa*, against which all three extracts showed a high activity.

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Functional analysis of an extracellular fructanase in *Lactobacillus crispatus*

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Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder. It affects 11% of the adult population in the developed countries and more in developing countries. Whole grain rye bread has been recommended as a good source of dietary fiber but its consumption may trigger IBS symptoms due to the content of fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) [1]. A low FODMAP diet allows IBS patients to manage their symptoms but also reduces the intake of dietary fiber [2]. The application of specific FODMAP-degrading sourdough to wholegrain bread produces a low-FODMAP bread without compromising the content of slowly fermented dietary fiber. *Lactobacillus crispatus* FUA3459 was isolated from a zero-FODMAP rye sourdough and carries a putative extracellular fructanase (FruA) which may be responsible for fructans degradation in rye sourdough. FruA or homologues, however, have not been characterized in lactobacilli [3].

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Effects of pecan shell walnut *(Carya illinoinensis)*, roselle flower *(Hibiscus sabdariffa)*, and red pepper *(Capsicum annuum)* powder on lipid oxidation and antimicrobial activity of beef patties during refrigerated storage

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The oxidation of some compounds in food, as the lipids, decrease the nutritional value and affect the organoleptic characteristics. The meat industry uses synthetic antioxidants (around the world), because are effective, cheaper and easy to obtain. Nowadays, the society demand more natural and healthy ingredients [1]. In this study, the antioxidant and antimicrobial activity of a by-product as pecan shell walnut (PSW) *(Carya illinoinensis)* and two plants as roselle flower (RS) (RS) *(Hibiscus sabdariffa)* and red pepper (CA) *(Capsicum annuum)* has been evaluated in beef patties, by different ways.

The antioxidant activity of their extracts assessed by DPPH and the total polyphenols was 30.12 mmol Trolox equivalents (TE)/g DW (PSW), 25.61 mmol TE/g DW (CA), 19.69 mmol TE/g DW (RS) and 36.14 mg of GAE /g DW (PSW), 30.21 mg of GAE /g DW (CA) and 22.40 mg of GAE /g DW (RS), respectively. On the other hand, the antimicrobial activity screening of PSW, CA and RS against Gram(+) and Gram(-) bacteria were achieved by agar disk-diffusion testing. The results showed that the RS had a high antimicrobial activity.

Beef patties treatments consisted of: (a) control (only meat), (b) 2% of PSW, (c) 4% of PSW, (d) 0.35% of CA, (e) 2% of RS, (f) 2% PSW with 0.35% of CA, (g) 2% PSW with 0.35% of CA and 2% of RS, (h) 4% PSW with 0.35% of CA and 2% RS, and (i) 0.7% of "CAMPA N.3 (Synthetic additive). All them were evaluated over a period of 12 days at 4±1 °C. At the day 7 the quantity of malondialdehyde per kg of meat in the control (a) was 2.3 higher than the sample (h) with 4%PSW with 0.35% of CA and 2%RS. At the same time, the % of metmyoglobin present in the control was almost twice that in the (h) sample. Microbial contamination has differences: the control in the day 5 presents more than 10^4 CFU/g sample; in contrast the samples (e) presents less than $3{\cdot}10^3$ CFU/g sample.

The results indicates that the treatment (h) with 4%PSW with 0.35% of CA and 2%RS could be used as natural food antioxidant/preservative. In addition, the sensorial trial indicates its acceptability.

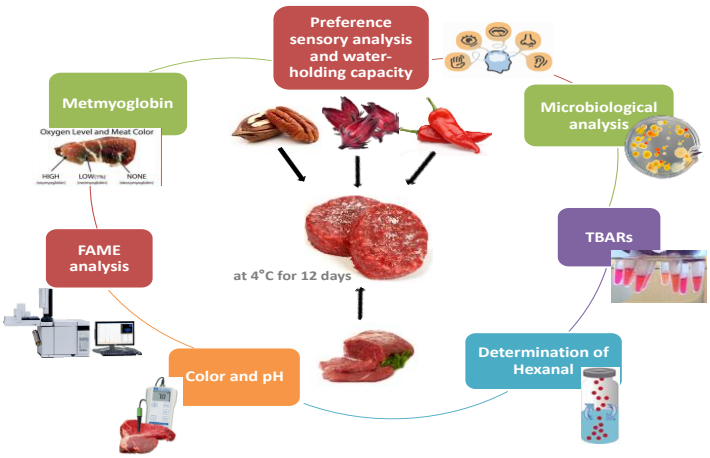

Fig.1. Methodology of the experiment

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Exploring the potential of USAMET/UHPLC-FLR methodology as innovative approach for determination of biogenic amines in fresh and caned tuna fish

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Biogenic amines (BAs), from which histamine, tyramine, putrescine, cadaverine, tryptamine, and β-phenylethylamine are the most common in plants and fermented foods, are organic nitrogenous compounds formed mainly by microbial decarboxylation of amino acids in protein-rich food. In spite of the low quantities of BAs present in foods, these compounds represent one of the most important risks for food quality and safety. Therefore, the development of rapid and sensitive methods for the determination of BAs is very important to the food safety and human health. However, the extraction and quantification of such compounds is challenging because foodstuffs are complex matrices and furthermore these compounds don't have fluorescence.

In this context, the aim of this work was the optimization and validation of an innovative analytical approach, based on ultrasound-assisted microextraction (USAMET) and derivatization with dansyl chloride (DnsCl) followed by ultra-high performance liquid chromatography with fluorescence detection (UHPLC-FLR), for a faster detection and quantification of the BAs tryptamine, cadaverine, putrescine spermine, histamine, tyramine and spermidine in fresh and caned tuna fish samples. Upon the optimization of different extraction and instrumental parameters, the best conditions achieved were a 5min-ultrasound (US) extraction with acetonitrile (ACN), followed by a DnsCl derivatization (3 mg mL-1, 10 min of US at 40 °C) and a chromatographic separation using a three-phase gradient (phosphoric acid (0.1%), methanol (MeOH) and ACN) with a flow rate of 200 µL min-1, with a total analysis time of 17 min.

Very satisfactory figures of merit were abtained for the method analytical performance with correlation coefficients (R2) higher than 0.9809, recoveries ranging from 76-106%, LODs ranging from 0.98 mg kg-1 (putrescine) to 8.57 mg kg-1 (tyramine)), and LOQs ranging from 3.20 mg kg-1 (putrescine) to 25.64 mg kg-1 (tyramine). Regarding the matrix, it was observed a significant influence on the extractive process for spermine and for tyramine. Overall, the methodology USAMET/UHPLC-FLR revealed an important improvement regarding the previous methodologies concerning the simplicity of the experimental layout, time of analysis and low amounts of reagents and samples.

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Sensory profile and phenolic compound composition of specialty coffees from three different regions

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Introduction: With an increasing demand for high-quality coffee, more attention is also focused on the impact of coffee on health. Specialty coffee is known as a high-quality sign, which means that from planting coffee tree till coffee brewing all processes are standardised to highlight the specific coffees origin characteristics. From the health point of view, phenolic compounds in coffee have proven antibacterial and antioxidant activity. There is limited research done on how the phenolic composition interferes with the sensory profile of specialty coffee from different regions. The aim of the current research was to evaluate the phenolic compound composition sensory profile of specialty coffee from three different regions.

Material & Methods: Three in Latvia roasted specialty coffees from different regions (Kenya, Colombia, Honduras) were selected. All samples were brewed using Fresh Press brewing technique before analysis. The volatile compound profile was analysed using gas chromatography method “Perkin Elmer Clarus 500” chromatograph with mass spectrometer. For individual phenolic compound determination high performance liquid chromatography „Shimadzu LC–20 Prominence” was used. Sensory evaluation was performed by Specialty Coffee Association (SCA) certified cupping team using the SCA cupping protocols.

Results: The highest chlorogenic acid content was detected in Honduras and Kenya coffees both of these coffees were processed using the washed method. Only in Honduras coffee compounds as sinapic acid, trans(3)-OH Cinnamic acid, quercetin and kaempferol were detected. Colombia coffee showed the highest catechin and epicatechin concentration. These compounds tend to have the highest concentration in coffee silverskin. Higher level of silverskin can be maintained using natural processing method. The sensory quality score increases with increasing coffee plantation altitude (MASL) but did not significantly influence the volatile compound composition and phenolic content. The sensory description of coffee samples matches with volatile compound odour characteristics. Compounds which are associated with fruity, floral odour characteristics (furfuryl acetate, 3-methyl-butanal, furfuryl formate) had higher peak area in Colombia coffee. Both Kenya and Honduras coffees showed 5–methyl–2–furancarboxaldehyde (red pepper odour characteristics) with high peak area.

Conclusion: The sensory quality score increases with increasing altitude. The processing method had the highest influence on specific phenolic compound concentration and volatile compound composition, but not on sensory quality score. Honduras coffee had the highest overall phenolic compound content, but Colombia showed higher catechin and epicatechin concentration and volatile compound profile with more fruity, floral odour characteristics. For further research, it is necessary to evaluate processing method influence to sensory profile and phenolic compound composition.

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Differentiation between three Iberian pig farms using fat from biopsies by means of stable isotopes, NIR, and fatty acids

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Spanish Iberian ham is one of the most beneficial meat products both within Spain and elsewhere. Its quality depends on the genetics of the pig, what it eats, and the technology used in the production of the ham. Ensuring the authenticity of this product is a major challenge and it is for this reason that the raw material, i.e. the animals, is monitored in the first place by the taking of samples of subcutaneous fat (biopsies) before the onset of the montanera (the period when the pigs feed on acorns, grass, and other natural resources of the dehesa pastureland). The characterization of the group of animals on each farm is important for the future knowledge of the products (hams and shoulders) which they will produce. The study presented here examines the composition of the fat from the pigs before the onset of the montanera.

This research is based on three farms, two containing 100% Iberian pigs and one containing crossbred pigs of Iberian mothers and Duroc fathers. All the determinants (stable isotopes, near infrared spectroscopy, NIR, and the lipid profile of fatty acids) are established from the fat of the biopsies. The samples are cleaned and the skin and any remains of meat or hair are removed. The biopsies from each farm (30) are classified in groups of 3 as the samples are not sufficient for obtaining all the determinants. Each biopsy is cut into two longitudinal parts with a scalpel; one of these is used for stable isotopes and the other for the NIR register and fatty acids. All the samples were kept at -30 ºC until used.

The analysis of stable isotopes has become a part of the European agrofood industry as it ensures the authenticity of products at their source. This analysis is carried out on an untreated sample that is cut up and homogenized by means of Politron equipment. In our case $\delta^{13}C$ were analyzed so as to trace the genetics of the animal and what it has eaten. The isotope ratios of $^{13}C/^{12}C$, which are used to calculate this parameter, maintain the digital fingerprint of the plants which have been used to feed the animal. The composition of $\delta^{13}C$ in ‰ vary from - 24.59 ‰ to - 26.35 ‰. The average data of the three farms were as follows: Farm 1 with 100% Iberian pigs - 26.19 ‰ $\pm$ 0.08; Farm 2 with 100% Iberian pigs - 25.37 ‰ $\pm$ 0.03; and Farm 3 with 50% Iberian pigs - 25.01 ‰ $\pm$ 0.05. These data reveal that by using stable isotopes it is possible to differentiate the three farms in a statistically significant manner. The NIR spectra are obtained from the biopsies by extracting the fat obtained in microwaves at a power of 1200 W and using position defrosting. To record the spectra we use cam-lok cups with an aluminum bottom and a path length of 0.1 nm with 15 microliters of extracted fat. Spectral differentiation is achieved by the RMS X residual method (calculating the square root of the arithmetic mean of the residual mean squares or RMS). From the spectral information, at the 1100-2000 nm interval with the SNV spectral correction the three groups of biopsies can be differentiated with 100% success in all samples.

The quantification of fatty acids by means of gaseous chromatography (GC) of the fat extracted from the biopsies by microwaves allows the determination of 30 fatty acids between C12:0 and C22:6. The values of palmitic acid, C16:0; stearic acid, C18:0; oleic acid, C18:1; linoleic acid, C18:2 n6, α-linolenic acid, C18:3 n3; Σ of saturated fatty acids, Σ of monounsaturated and polyunsaturated fatty acids give us a different lipid composition for each farm.

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Use of alternative protein sources in wafer production

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Cereals and bakery products provide a significant proportion of our daily carbohydrate and protein needs. The most important raw material for the baking industry is wheat, which, besides its favourable physiological effect, is one of the most common sources of allergens among celiac patients. Nowadays, there is an increasing interest in fortification of baked goods by replacing wheat flour with non-wheat flours. The aim of this study to prepare gluten-free flour mixture which has higher protein content than the wheat flour, provide valuable source of protein and essential amino acids for human nutrition and is suitable for bakery use. The wafer samples were made from millet flour, which was supplemented with hemp, alfalfa and lupine.

The amino acid composition of the flours was determined by Ingos 400 Automata Amino acid Analyser and Protein Digestibility Corrected Amino Acid Score (PDCAAS) was calculated using reference data. Lysine is the limiting amino acid of millet (15.1 mg/g protein, PDCAAS 0.3) and of hemp (36.9 mg/g protein, PDCAAS 0.67). Methionine+cysteine are the limiting amino acids of lupin (12.9 mg/g protein, PDCAAS 0.51) and of alfalfa (19.9 mg/g protein, PDAAS 0.74). The optimal flour mixture were calculated on the essential amino acid content of the different flours taking into account their PDCAAS values. Using legumes such as lupin or alfalfa in combination with millet flour improves the PDCAAS scores of the blends. The protein contents of the wafer samples were 9.4-12.0 g/100g contributing more than 12 energy% of total energy. Based on both protein quality and quantity of the wafer samples, the addition of different flours improves the balance of the essential amino acids, thereby this reformulated product can be qualified for a “Good Source of Protein” classification.

The hardness of the different wafer samples were measured by Stable Micro Systems TA.XT2 Texture Analyser equipped with a Light Knife Blade fixture. It has been established that wafer prepared from flours containing 2:1 (w/w) millet:hemp was the hardest (1226 g) and appealingly crispy product. Crustiness of the product decreased (889 g) by adding lupine and alfalfa to the flour (millet:lupine:alfalfa 2:1:1). Sample prepared from flours containing 1:5 (w/w) millet:alfalfa was the softness (517 g).
This initial study provides the basis for further research to obtain optimal flour compositions for gluten free wafer.Gluten-free eating often involves limitation, but we believe that wafers are the product that can be very enjoyable without gluten!

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Studies on the oxidative stability of beers by Electron Spin Resonance (ESR)

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Packaged pasteurized beers have a shelf life which is essentially determined by appearance of haze or by the deterioration of the flavour. These are the result of oxidation phenomena entailing active oxygen species, such as H_2O_2, $HO^{\bullet}$ and $HOO^{\bullet}/O_2^{\bullet-}$. Oxidation reactions are based in Fenton reactions which involve oxygen and iron or copper ions. Then, minimizing the concentration of these species in packaged beers must have a positive effect on stability of beer [1, 2].

In beer, oxygen plays an important role during the brewing process causing a rapid deterioration of beer flavour. The reactive species of oxygen formed during the Fenton reaction can react with other molecules present in matrix which can decrease the beer stability. Hydroxyl radicals, for example, are involved in beer oxidation because they are one of the most reactive species that can react with ethanol [3]. The addition of antioxidants, such as sulphite, can inhibit these reactions.

This work aims evaluating the influence of the storage conditions, mainly the temperature and shelf life, on the oxidative stability of beer. Beers were exposed to natural and forced aging (6 months at 20ºC and 2 weeks at 37ºC, respectively) and the oxidation stability was evaluated by Electron Spin Resonance (ESR). Hydroxyl radicals are mainly trapped by ethanol, forming 1-hydroxyethyl radicals. The quantification of these radicals was performed by using the spin trap *n-tert*-Butyl-α-phenylnitrone (PBN), which forms a stable spin adduct with the mentioned radicals.

The formation of radicals in beer was evaluated based on the studies by Andersen et al. [1]. Beer (25 mL) containing PBN (30mM) was heated at 55ºC in a water bath. Samples were transferred directly into a capillary quartz tube and ESR spectra were recorded. To quantify the amount of spin adducts the maximum intensity and the minimum intensity of the central doublet of the spectra was measured. The effect of different concentrations of sulphite on the amount of spin adducts was also investigated.

Slight differences were observed for the obtained lag phase between beers exposed to forced aging and beers maintained at 4ºC. However, the main differences were found for the naturally aged beers (6 months, 20ºC) in relation to control beers (4ºC). Beers that are exposed to natural aging did not present a lag phase, and the quantity of spin adducts increases after 45 minutes of reaction with spin trap.

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"*Beta vulgaris* L." leaves and stalks - Separation and purification of bioactive compounds using membrane technology

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The beet "*Beta Vulgaris L.*", of the *Chenopodiaceae* family, is a vegetable with high antioxidant activity, proven to prevent cardiovascular diseases and control dyslipidaemia [1,2,3]. These features were seen to be directly related to the polyphenols (PCs) present in this vegetable [2,3]. However, only the root of beet is typically consumed, and although its leaves and stalks are rich in bioactive compounds they are often considered agricultural residues [2]. The aim of this study is to evaluate the levels of phenolic compounds and antioxidant properties of the aqueous extracts of beet leaves and stalks, and the ability of the membrane technology in separating and purifying this extract.

In this work, to avoid the use of organic solvent and to simulate the intake, several aqueous extracts from beet leaves and stalks were obtained: Decoction (Bdec), partial enzymatic digestion with pepsin (BDG), partial enzymatic digestion with pancreatin (BDP) and complete enzymatic digestion with pepsin and Pancreatin (BDC). The extracts were characterized by quantifying its total phenols (Folin-Ciocalteu method), proteins (Bradford method), carbohydrates (Anthrone method), and the biological activities were assayed, namely antioxidant activity (DPPH assay) and the inhibitory activity of enzyme Acetylcholinesterase (AChE). In the end the BDP extract has presented the best results showing a 0,554 mg/µL of total phenols, and an IC 50% of the assay of antioxidant activity and inhibitory activity of enzyme AChE of 0,210 mg/µL and 0,375 mg/µL of total phenols, respectively.

Additionally, a separation methodology was developed for the purification of bioactive compounds from BDP extract using membrane technology by ultrafiltration in concentration mode [4]. In this stage, five cellulose acetate membranes were prepared through the phase inversion method covering a wide range of Molecular Weight Cut-Offs (MWCO) between 3,5 kDa and 62 kDa. The permeation experiments with the BDP extract were carried out at three transmembrane pressures (1, 2 and 3 bar). The concentrate and permeate streams of the BDP extract from the five membranes were characterized and biological activities assayed. It was also found that the permeate of the five membranes obtained at the transmembrane pressure of 3 bar presented a higher quantification of total phenols. But the permeate obtained from the membrane with MWCO of 4,8 kDa and the transmembrane pressure of 3 bar presents higher ability to recover bioactive compounds with an IC 50% of the assay of antioxidant activity and inhibitory activity of enzyme AChE of 0,188 mg/µL and 0,267 mg/µL of total phenols, respectively.

In this work, a high value agriculture residue, such as beet leaves and stalks, was seen to have advantages to be incorporated in diet, displaying valuable health properties. When the aqueous extract is processed using membrane technology, many active bioactive compounds can be obtained. which ultimately can be used to supplement diets or incorporate food products, such as pasta, juices or sauces [3].

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How to increase the potential of an invasive plant? The case of *Gunnera tinctoria*

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Gunnera tinctoria, also named Nalca, is a South America native plant. It exists in Azores but only in the São Miguel Island. Being a highly competitive plant, *G. tinctoria* is able to colonize and threaten the native Azorean forest [1]. In Chile, this plant is used in different ways: to prepare root infusions, to cook traditional dishes (leaves and petiole), and to dye wool and cotton [2]. Due to its pharmaceutical properties, *G. tinctoria* is also used in folk medicine to treat diarrhea, genitourinary infections, digestive diseases [3] and fever [4].

The aim of this work was to compare the nutritional and antioxidant profiles of the different parts of *G. tinctoria* from São Miguel, Azores, evaluating the potential of this plant as a novel food or ingredient, simultaneously contributing to its dissemination control.

The samples were collected in Sete Cidades, São Miguel Island, in September of 2018. For better preservation, after separation, the different parts of the plant (flowers/stems, baby leaves, adult leaves, and roots) were lyophilized. The nutritional profile (moisture, ash, total lipids, total protein, and total dietary fibre - soluble and insoluble) was determined using AOAC official methods [5]. Available carbohydrates were calculated by difference. Total phenolic and total flavonoid contents, as well as the antioxidant activity (using the ferric reducing antioxidant power (FRAP) and the DPPH$^{\bullet}$ inhibition assays) were evaluated using spectrophotometric procedures [6].

The results show that the adult leaves contained the highest levels of total dietary fibre (44%, 95% insoluble fibre). The roots were the richest in available carbohydrates (80%) and total flavonoid compounds (30 mg catechin eq./g). The baby leaves presented the highest values for: total phenolics (176 mg gallic acid eq./g), ferric reducing power (13 mmol ferrous sulphate eq./g) and DPPH$^{\bullet}$ scavenging ability (705 mg trolox eq./g).

In conclusion, the adult leaves are a good source of fibre, being suggested as an alternative ingredient to increase the insoluble fibre of foodstuffs. In addition, the roots seem to be an excellent source of energy, for instance, to be incorporated in energetic supplements. Finally, the consumption in salads or infusions of the baby leaves, the richest in antioxidants, is also an option.

Acknowledgments:
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Quality of Iberian products: composition of fatty acids and stable carbon isotopes correlations in subcutaneous fat

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Iberian ham is one of the most highly prized products of Spanish gastronomy. The prior monitoring of subcutaneous fat when the Iberian pig is slaughtered allows us to predict the quality of final products such as hams and shoulders. The composition of the fatty acids of the subcutaneous fat when the Iberian pig is slaughtered is an indicator of the *montanera* diet given to the Iberian pigs. On the other hand, the isotope ratio $^{13}C/^{12}C$ expressed as $13\delta^{13}C(‰)$ allows the reconstruction of the diet owing to the fact that animal tissues retain the trace of the plants on which they have fed. In this way it is possible to distinguish between animals reared on intensive farms which are fed on C4 plants (mainly maize) and livestock fed exclusively on grass and acorns (Schmidt et al., 2005). The addition of fodder simulating a composition resembling that of the *montanera* makes it necessary to search for other methods, which although they are costly such as the analysis of stable isotopes are decisive in predicting the quality of the raw material. The objective of this study was to seek correlations between the fatty acid composition and the isotope ratio of fat samples.

In order to carry out the project samples (106) of subcutaneous fat from the area of the coccyx, from both 100% Iberian pigs and 50% Iberian pigs at the time of slaughter and after varying periods on the *montanera* pastureland (70, 85, and 120 days) were analysed.

The Pearson correlation coefficient between the fatty acids and the value of $13\delta^{13}C(‰)$ were calculated using SPSS software. The more negative the value of the isotope ratio the greater the correlation with feeding based on C3 plants such as acorns and grass. We found statistically significant correlations with a high absolute value between more negative $13\delta^{13}C(‰)$ value and higher contents of oleic acid C18:1, linoleic acid C18:2 n3, α-linolenic acid C18:3 n3, heneicosanoic acid C21:0, erucic acid C22:1 n9, and arachidonic acid C20:4 n6, together with the S of polyunsaturated and monounsaturated fatty acids. On the contrary higher contents of palmitic acid 16:0, stearic acid C18:0, ɣ-linolenic acid C18:3 n6, tricosanoic acid C23:0, and the S of the total of saturated fatty acids show significant correlations with less negative values of $13\delta^{13}C(‰)$ (fatty acids found in a lower proportion in acorns).

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Application of NIR Spectroscopy in Classification of the Iberian pig

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The Iberian pig is a breed native to Spain which is particularly suitable for producing cured ham one of the most highly appreciated meat products both in Spain and elsewhere. The main characteristic of Iberian pigs is the specific form of distribution of the fat and their capacity to infiltrate the fat between the muscle fibers. This characteristic is an essential element of the palatability of the meat, facilitating the separation of the muscle fibers and improving the sensation of the tenderness of the ham. The Iberian pigs used in the production of hams are classified depending on the race purity in "100% Iberian" coming from pure-race parents and 75% or 50% "Iberian" which are partly Iberian mixed with Duroc race father. As far as the feeding of the pigs is concerned, products from animals slaughtered immediately after feeding exclusively on acorns, grass, and other natural resources of the *dehesa* pastureland, may be denominated "*bellota*". In the case of animals fed on fodder and acorns may be denominated "*cebo de campo*" and if the feeding is exclusively based on fodder they are denominated "*cebo*".

Certain non-destructive techniques have been widely assessed in relation to their implementation in the production of dry-cured ham, such as Near Infra-Red Spectroscopy (NIRS). This technique is easy to use, accurate, and robust and can be implemented without problems in the industry by using NIR on-line instruments. NIR spectroscopy has been shown to be a fast and efficient tool for assessing the quality of the meat and has a high potential for predicting the chemical properties and classifying it according to its quality. In relation to dry-cured ham, NIR has been successfully used in the classification of hams in relation to the defects of pastiness and anomalous coloration, in the development of prediction models of the a_w content and the NaCl content, and in the prediction of sensory attributes such as texture and color.

In this study, NIR Spectroscopy coupled with a remote reflectance fiber optic probe has been used. The analysis was carried out by applying the probe directly on a slice of ham and the spectrum were recorded in the 1100-2000 nm interval. The aim was to discriminate the feeding system and the breed of the animals. To do this, samples from 63 controlled hams were analyzed: 13 samples correspond to 100% Iberian "*bellota*" hams" and 50 samples correspond to 50% Iberian hams 25 of these were "*bellota*" hams and the 25 remaining were "*cebo*" hams. Spectral differentiation is effected by means of the residual RMS X method with the SNV correction, 2-4-4-1. An equivalent number of samples were taken at random depending on the factor to be differentiated in each case (13 samples for differentiation by breed and 25 for differentiation according to food). The model allows the correct classification of 100% of the "*bellota*" hams and 96% of the "*cebo*" hams. As far as the breed is concerned, 100% of the hams were correctly classified, which shows that NIR spectroscopy allows the differentiation of hams from 100% Iberian pigs and hams from crossbred pigs. The results of this study show that NIRS could be used in hams to identify both the breed and the feed of the animals of origin. These preliminary conclusions should be confirmed by analysing a larger number of samples.

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Lactic acid fermentation of cereal by-products: an aroma enhancer tool

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Cereal by-products represent the mainly side-stream of milling plants. These heterogeneous materials are considered residues and consequently discarded, resulting in a not negligible loss of valuable material. Nowadays, several innovative techniques are applied to food by-products with the final aim to recover and valorise in a sustainable way such resources. One of these is the lactic acid fermentation, which uses the metabolism potential of lactic acid bacterias (LAB) in order to modify the overall characteristics of the food matrix [1]. The aroma profile of food is an aspect that plays an important role on the final consumer acceptance of a products [2].

In this study, the ability to bio-transform or generate novel aroma compounds of LAB in industrial maize germ and rice bran were studied. The solid-state fermentation (SSF) was performed on the autoclaved raw materials in order to ensure the sterility of the products using *Lactobacillus rhamnosus* 1473, a dairy origin LAB strain [3]. SSF was prolonged until 48 hours, and sampling occurred at time zero and after the autoclaving step. The analysis of volatile fractions was conducted using a head space solid phase micro extraction gas chromatography coupled to a mass spectrometer (HS-SPME-GC-MS) technique and using toluene as internal standard for the semi-quantification.

A total of 65 compounds were identified, belonging to alcohols, aldehydes, ketones, esters, carboxylic acids and other compounds. Then, 1-hexanol and 2-undecanol were probably synthesised during storage, since they generated during degradation of hydroperoxides [4]. On the contrary, compounds like 1,4-Butanediol and 2-ethyl-1-hexanol were found in fermented raw materials only, thus produced *ex-novo* by microorganism.

In conclusion, we could confirm the potential of LAB as maize germ and rice bran aroma profile enhancer, capable to shift a green/herbal and fatty flavour to a floral and fruity perception with strong acidic note (Fig.1). However, a stability study must be conducted in order to determine the effective permanence of the novel compounds. Beside this, SSF of agro-industrial by-products appears to be a useful technique for their total recovery.

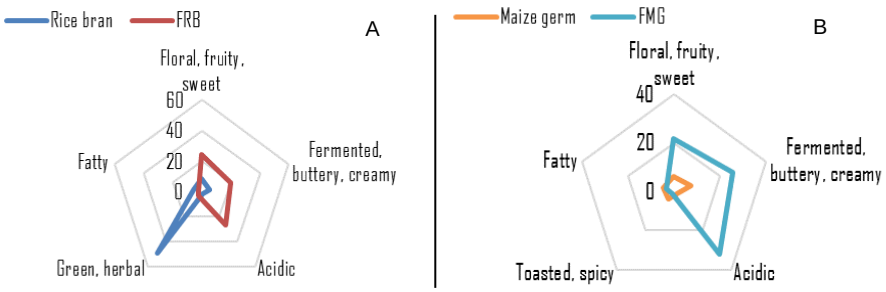

Fig.1. Spider webs depicting the mainly odour types (OT) modifications of raw and fermented rice bran (FRB) (A) and maize germ (FMG) (B). Unit expressed as sum of mg/kg of each compound with the same OT.

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Discrimination of geographical origin of oranges (Citrus sinensis L. Osbeck) by ^{1}H NMR fingerprinting and multivariate statistical analyses

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An untargeted method using ^{1}H NMR fingerprinting in combination with chemometrics was developed for the discrimination of oranges of three geographical origins (Italy, South Africa and Spain). Three multivariate statistical models, i.e.PCA/LDA, SELECT/LDA and PLS-DA, were built and relevant performances were compared (Table 1). The obtained results were found to be satisfying for all the models; in particular, SELECT/LDA provided the highest prediction abilities in cross-validation and external validation with mean values of 100.0% and 97.9%, respectively. These results indicate that the proposed NMR method in combination with multivariate statistical analysis provides an effective tool for authentication of the geographical origin of oranges Subsequently, with the purpose to get information about the potential metabolites responsible for the geographical discrimination of oranges, VIP values were used to identify the compounds most useful for this aim (VIP>1.5), and it was highlighted that the most contributing coumpounds were sucrose, quinic acid, alanine, ethanol, proline, dimethyl pimelimidate (DMP), citric acid, succinic acid and other unidentified compounds. Moreover, to confirm the importance of these metabolites, the 9 identified compounds were also investigated by one-way ANOVA analysis. The content of all molecules resulted significantly different among samples belonging to three classes ($p < 0.05$). In particular, it was possible to note that ethanol, DMP, sucrose and alanine allowed to discriminate Spanish oranges from the samples of the other two classes while citric acid discriminated Italian fruits from the Spanish ones but the confidence interval of citric acid seemed to be partially overlapped between African and Spanish classes. No other compound was able to discriminate Italian samples from the other two classes.

Table 1. Recognition and prediction abilities for the PCA/LDA, SELECT/LDA and PLS-DA models classifying oranges according to their geographical origin.

	Model performance (%)											
	Recognition ability (Modelling)				**Prediction ability (CV 10)**				**External Prediction**			
	ITA	**AFR**	**SPA**	**MEAN**	**ITA**	**AFR**	**SPA**	**MEAN**	**ITA**	**AFR**	**SPA**	**MEAN**
PCA/ LDA	100	100	93	98	97	100	93	97	84	100	100	93
SELECT/ LDA	100	100	100	100	100	100	100	100	95	100	100	98
PLD-DA	97	100	93	97	97	100	93	97	89	100	100	95

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Influence of cardoon and time phase of cheese production in PDO Serra da Estrela cheese: case study

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Serra da Estrela (SE) cheese is a traditional agri-food product with Protected Designation of Origin (PDO), manufactured at the farm level only from raw ewe's milk (exclusively from Serra da Estrela and Churra Mondegueira sheep breeds), coagulated with flowers extract of the cardoon flower (*Cynara cardunculus* L.) and salt. The cheese organoleptic and physicochemical properties are affected by many parameters, from the characteristics of used ingredients to processing factors. The cardoon flower is the agent responsible for the milk coagulation, due mainly to the activity of two aspartic proteases, cardosins A and B [1, 2]. In addition to its clotting ability, it has a strong proteolytic activity that leads to an extensive breakdown of caseins in the cheese matrix, contributing to the unique features of the final product [3]. Another factor that account for the high variability of cheese final attributes is milk. The chemical characteristics of bulk milk change along the milking season, being influenced by the flock lactation period and ewe's feed (natural pastures or commercial feed) [4].
The purpose of this work is to study the cardoon flower influence (one cardoon previously characterized [5] and one commercially available) and time phase of cheese production (November and February) in the final characteristics of PDO SE cheese. The parameters analysed were moisture, protein, fat and salt contents, texture and colour. Texture Profile Analysis (TPA) was performed to assess hardness, springiness, cohesiveness, gumminess and resilience. Colour was evaluated using the CIELAB colour space (CIE L*a*b*).
The two-way ANOVA test employed for comparison of means consider cardoon type, time phase of cheese production and their interaction as independent variables. The test was applied with SPSS v25 with a 5 % of significance. During the milking season it was registered an increase in the fat content and a decrease in the protein content in the analysed cheeses ($p<0.05$). Regarding the rind texture and colour parameters, cheeses produced in February were lighter and more yellow than the ones produced in November ($p<0.05$), which presented higher values for hardness and gumminess ($p<0.05$). Concerning cardoon flower influence, the commercially available produced a softer and less gummy cheese rind ($p<0.05$), with higher fat content ($p<0.05$) and greenish colour ($p<0.05$). The interaction between the two independent variables influenced significantly the fat content and the rind and paste texture ($p<0.05$).
Thus, the cardoon type and time phase of production are crucial for the final characteristics of PDO SE cheese, mainly in terms of rind colour and texture, and fat content. Future studies will include the analysis of cheese produced in more than two-time phases, comprising the end of the milking season.

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Use of seed defatted flours to elaborate gluten-free cookies

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Cookies are referred as baked products containing three major ingredients: flour, fat and sugar. They represent the largest category of bakery snacks due to their low cost, good taste, texture (crispness) and storability, and are considered an effective vehicle of nutrient supply to consumers. However, cookies are generally made with wheat flour due to its unique visco-elastic properties, but it may originate allergic reactions to coeliac people.

To solve this problem, and intending to increase the nutritional value of cookies, wheat flour was substituted by defatted flours from flax, sesame, chia and poppy, which are by-products of the oil extraction industry. From the physical point of view, clear differences were observed in the colour of the cookies, which are related to the colour of the defatted flours. Regarding texture, wheat cookies used as control showed a more elastic behaviour than the cookies elaborated with defatted seed flours, which showed a higher tendency to crumble. The use of flax, sesame, chia and poppy defatted flours improve the nutritional properties of cookies, with an increase in protein (>15 % in all cases), fibre and fat content, but with a decrease in total carbohydrates. The increase in fat content is due to the remaining oil in the defatted flours, which may reach values about 20 % when pressure systems are used. Anyway, seed oils show positive characteristics as they are rich in polyunsaturated fatty acids, and this oil can be considered also a healthy source of fat.

From the sensory point of view, the cookies elaborated with flax and sesame defatted flours showed similar values than the wheat cookies used as control regarding all the parameters evaluated (colour, smell, taste, texture, appearance and global acceptability). Thus, the use of defatted seed flours is proposed as an interesting alternative to improve nutritional properties of cookies.

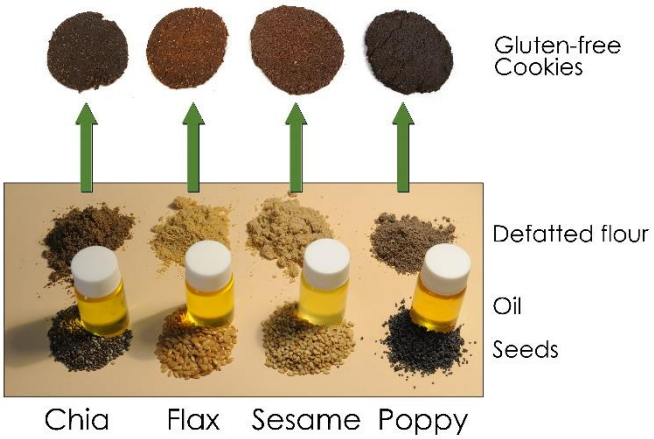

Fig.1. Defatted flours are a by-product from the oil extraction industry, which may be used to elaborate gluten-free cookies

Assessment of nutritional composition, physicochemical properties and bioactive compounds of two strawberry cultivars produced in Western region of Portugal

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In Portugal, strawberry production has been increasing due to high acceptance of consumers for its sensory attributes and bioactive compounds with beneficial properties recognized for consumer health. Strawberry play an important role in Mediterranean Diet, because it´s a rich source in phenolic compounds specially anthocyanin's with high antioxidant capacity. The antioxidant activity of phenolic compounds is related to the ability of these compounds to absorb free radicals, thus preventing the occurrence of oxidation reactions leading to cellular damage, which are responsible for cell damage. However, strawberry during the growth period is subject to several factors that compromise the nutritional and bioactive content of fruits, such as agricultural practices, production region, climatic conditions as well as cultivars. In order to increase the strawberry consumption, it´s necessary to ensure that fruits produced in Portugal are higher regarding overall quality with phytochemical properties.
The main goal of the present study was to evaluate the nutritional composition (protein, lipids, carbohydrates, ash, moisture [1]), physicochemical properties (soluble solids content: SSC, titratable acidity [2] and texture and instrumental colour of fruits surface and interior) and bioactive content (total phenolic: TPC [3], DPPH radical scavenging capacity [4], ferric reduction capacity: FRAP [5], anthocyanin content: AC [6]) of two strawberry (Fragaria x ananassa Duch.) cultivars ('Endurance' and 'Portola') produced in Western Region of Portugal (Caldas da Rainha). From the obtained results, no significant differences ($P > 0.05$) was detected in nutritional composition of both strawberry cultivars, with exception of protein content observed in cv. 'Portola' (0.57 g.100g-1 ± 0.04) compared with cv. 'Endurance' (0.72 g.100g-1 ± 0.05). The cv. 'Endurance' showed as sweeter, redder and less firm ($P < 0.05$) than cv. 'Portola'. Also, significant colour changes ($P < 0.05$) was observed in a* and b* colour parameters of strawberry cultivar pulp and surface, respectively. Comparing the bioactivity of strawberry cultivar, cv. 'Endurance' revealed the highest content as shown in Table 1.
Overall, strawberry cultivar 'Endurance' and 'Portola' showed an interesting nutritional value and phytochemical composition that is relevant for the Mediterranean Diet. In addition, the potential bioactivity of both fruits cultivar should be studied for related consumer health benefits.

Table 1. Phytochemical characterization of two strawberry cultivars produced in Western region of Portugal (average ± standard deviation)

Strawberry Cv.	TPC (mg GAE.100g^{-1})	DPPH (%)	FRAP (mmol Fe_{2+}.Kg^{-1})	AC (mg.100g^{-1})
'Endurance'	1312.50^{a} ± 0.01	51.07^{a} ± 5.22	0.72^{a} ± 0.08	2.41^{a} ± 0.01
'Portola'	603.75^{b} ± 0.01	41.97^{a} ± 2.34	0.38^{b}± 0.01	2.18^{b} ± 0.02

Within a column, different letters represent significant differences at p <0.05.

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Disposable miniaturized device for DNA purification from complex samples

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With the increasing complexity of the food supply chain, issues related to food safety and quality control are major concerns not only for food authorities and food industry but also for the consumers themselves. Consumers are more aware of these issues and increasingly demanding reassurance on the authenticity of their food, regarding content and origin. A variety of analytical methods have been developed and extensively tested for food authenticity applications [1,2]. However, DNA-based technologies have been rapidly complementing or, in some cases, replacing these methods, being not only applied to food authenticity and traceability but also for identification of genetically modified organisms (GMOs), detection of allergenic ingredients and detection of foodborne pathogens [3-5]. Despite of its advantages, DNA-based analysis often requires specialized equipment, costly reagents, highly trained personnel and large sample sizes, limiting its applicability as a routine analysis. Therefore, miniaturized DNA analysis devices for *in situ* applications are being developed either with a modular approach, focusing in each step separately, or with an integrated approach, including all steps in a single device. This miniaturization brings several advantages over conventional methods, such as the smaller volumes required, lower cost, portability and improved performance, being faster and more sensitive [6]. DNA extraction and purification is a critical step of DNA analysis, which should ensure an efficient recovery of nucleic acids and at the same time the removal of inhibitors that might affect the efficiency or even completely inhibit the amplification of the target DNA sequence.
At INL, our research group is working on the development and optimization of tailored, miniaturized and automatized devices to perform DNA analysis from complex food samples in a modular approach. A washable and reusable miniaturized device for DNA purification has already been designed and optimized, using a highly efficient protocol for olive oil, which is considered as one of the food products most at risk of food fraud [7]. We are currently working on the development and optimization of a miniaturized disposable device for DNA purification. This new device includes a chamber with micropillars closely arranged and functionalized for a pH-induced DNA capture and release. This protocol has important advantages, such as providing aqueous DNA extraction on a microchip, thus not involving the use of chaotropic/organic reagents, and at the same time delivers DNA in a purified, PCR-ready form [8], which is of high importance when developing micro total analysis systems (μTAS). The protocol has been optimized regarding buffer composition, pH, collected volumes, time and applicability for automatization. The developed protocol shows high DNA recovery and its application to complex matrixes such as challenging food samples is being evaluated.

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Rheological and Physicochemical Properties of Portuguese commercial Honeydew Honey

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Honeydew honey is an unconventional type of honey which has been attracting an increasing interest by the Food Industry and consumers, due to its distinct chemical and physicochemical features. The differentiated properties (higher pH, lower content of monosaccharides, higher contents of di- and trisaccharides, darker color and higher content of phenolic compounds and protein), when compared with most blossom honey, concur to a remarkable bioactivity (1,2). Literature studies have underlined the therapeutic potential of honeydew honey, therefore requiring a careful physicochemical characterization.

Rheological properties are relevant in all stages of honey production, depending on chemical composition and moisture content, among others factors, and being related to quality control and sensory profile (3).

For the characterization of Portuguese commercial honeydew honey purposes, some physicochemical (pH and acidity, water content, color, electrical conductivity, reducing sugars and apparent sucrose content), antioxidant (total phenolic content, ferric-reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazil free radical scavenging ability and oxygen radical antioxidant capacity) and steady/dynamic rheological (viscosity, elastic modulus and loss modulus) parameters were determined.

The physicochemical characteristics of analyzed honeydew honey were within the expected parameters for this type of honey. Additionally, it expressed a significant antioxidant capacity, which showed to be strongly dependent on polyphenols.

Honeydew honey exhibited a Newtonian behaviour. Elastic modulus (G´) and loss modulus (G´´) were both influenced by oscillation frequency. Both parameters were subjected to the Power-law model, and the viscous intercept (K´´) showed a magnitude much greater than that of the elastic intercept (K'), confirming the liquid-like behaviour of honeydew honey.

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Transformation of sugars in carbonated soft drinks and syrups

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The use of different kinds of sugar sweeteners, especially sucrose and high-fructose syrups reflects the differences in tradition, availability, economy, and technological background in the production of carbonated soft drinks. The presence of free fructose in foods and drinks leads to frequent speculations on different sweetness, contribution to higher obesity and profiles of satiety. Therefore, the use of alternative sugar sweeteners can lead to different sensory, nutritional as well as physiological and toxicological properties of carbonated soft drinks.

During storage, carbonated (acidic) soft drinks undergo sucrose inversion and further conversion of the monosaccharides. The principal intermediates and products of non-enzymatic degradation of reducing carbohydrates are reactive carbonyl species (RCS) such as alfa-dicarbonyl compounds (alfa-DC), incl. 3-deoxyhexosulose (3-DG) and methylglyoxal, and 5-hydroxymethylfuran-2-carbaldehyde (HMF) [1-3]. Their presence in foods is associated with certain risks, such as mutagenic activity and damage to macromolecules by means of glycation mechanisms, although some positive effects are also attributed.

In this work, RCS levels and kinetics of their formation/degradation were evaluated in soft drinks of the cola type with different sugar sweeteners during short (days to weeks) and long-time storage (months to years). Besides that, we examined the syrups from the soft drink production and syrups used as culinary sweeteners and nutraceuticals. In addition to the soft drinks and syrups sampled immediately after production, we analysed also model soft drink systems with a particular sugar.

The differences in RCS content among the soft drinks of the same age with fructose syrups (HFCS with 55% fructose), glucose syrups and sucrose are significant. They result from different kinetics of the formation of alfa-DC and HMF. The initial level of alfa-DC in fresh beverage is determined by the concentration of alfa-DC in HFCS used for the production of soft drink. In carbonated soft drinks, fructose is a better precursor of RCS than glucose and much better than sucrose, thus fructose is a key factor for the formation of RCS. Fresh carbonated soft drinks with different sugar sweeteners differ significantly in 3-DG levels, while those several weeks-to-months old are likely to differ in HMF levels and methylglyoxal is a good marker of their long-term storage.

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Nutritional composition of four varieties of *Opuntia ficus-indica* (L.) Miller cladodes

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Opuntia ficus-indica (L.) Miller is a native cactus from South America. This species arrived to Europe at the time of the discoveries and has expanded to places with more favorable conditions to its growth [1]. In Portugal, it was mainly in the Algarve and Alentejo regions. Since 2009, intensive and orderly cultivation of this cactus gained more expression for fruits production [2]. Therefore, the availability of cladodes – the cactus stems – has been increasing, becoming a concern for producers due to their highly invasive potential [3].
Cladodes are not part of the Portuguese food pattern, but their recognition and valorization as food can contribute to food security. In that perspective, this study aimed to assess the nutritional composition of different varieties of the *Opuntia ficus-indica* (L.) Miller cladodes, from Portugal (Torres Novas). The samples used were cladodes from plants that produces white, orange and red ficus. The white varieties were obtained from cactus orchards in *Arrepiado* and *Barquinha* and orange and red varieties were obtained in *Olaia*, in December of 2018.
Samples moisture was evaluated using an infrared balance. Total fat, protein, ash, and dietary fibre were assessed accordingly to AOAC official methods [4]. Available carbohydrates content was calculated by difference. The results were expressed as g/100 g of dry weight.
The nutritional value of the different varieties of Cladodes studied is presented in Table 1. Overall, cladodes presented low fat and high dietary fiber contents. Regarding dietary fibre, the sample from *Arrepiado* presented the highest amount, followed by orange of *Olaia*, white of *Barquinha*, and red of *Olaia.*
Given the nutritional importance revealed in this study, it is suggested the use of cladodes in food products, promoting diversity and nutritional value.

Table 1. Nutritional composition of four varieties of cladodes

Sample	Moisture (%)	Fat*	Protein*	Ashes*	Dietary Fibre*	Carbohydrates*
White of Arrepiado	8.05 ± 0.39	1.17 ± 0.03	4.90 ± 0.37	17.21 ± 0.22	36.78 ± 1.02	40.11 ± 0.03
White of Barquinha	8.39 ± 0.21	1.24 ± 0.02	7.62 ± 0.77	20.35 ± 0.15	24.23 ± 0.02	47.21 ± 0,28
Orange of Olaia	7.59 ± 0.06	1.30 ± 0.01	4.14 ± 0.37	26.99 ± 0.72	32.90 ± 1.25	35.14 ± 0.55
Red of Olaia	10.63 ± 0.35	1.73 ± 0.33	3.48 ± 0.36	20.70 ± 0.04	20.51 ± 0.20	53.62 ± 0.59

** Results expressed as g/100 g dw (mean ± standard deviation).*

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Cinnamomum burmannii hot water extract: a flavouring and thickening ingredient

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Cinnamon (*Cinnamomum*) is a very well known spice, widely used as a flavouring agent in foodstuffs. There are a number of so called cassia varieties that are used, along with *C. zeylanicum*, called the verum cinnamon. Despite its commercial availability, *Cinnamomum burmannii* (Padang cassia) hot water extracts originate high viscous aqueous solutions (1545 cP) in relation to verum (51 cP), which prevent a broader use by food industry.

Following the hypothesis that cinnamon polysaccharides are the responsible for the viscous properties of the solutions, in this work, the soluble polysaccharides from Padang cassia were structurally characterized by sugar and methylation analyses [1], using *C. zeylanicum* (verum) for comparison. Cinnamon cassia water extract is mainly composed by arabinose (51 mol%) and xylose (34 mol%), whereas cinnamon verum has higher content of glucose (28 mol%) and uronic acid (27 mol%) with lower content of Ara (24 mol%) and Xyl (14 mol%). The linkage analysis of cassia polysaccharides revealed the presence of highly branched arabinoxylans constituted by a backbone of 4-linked xylans branched with terminally-linked Ara and Xyl at C2 and/or C3. Verum showed also to be constituted by highly branched arabinoxylans concomitant to the presence of 4-linked glucans. Based on these analyses, an acidic treatment with 0.65% (w/w) citric acid during 90 min at 90 ºC was performed, replacing the hot water extraction. This treatment allowed to reduce the viscosity of the aqueous solution to 80 cP, a value approaching the viscosity obtained for cinnamon verum.

The volatile composition of cassia and verum cinnamons, as well as of cassia after acid hydrolysis treatment, was analysed by head-space-solid phase microextraction (HS-SPME)/ GC-qMS [2]. In all samples, cinnamaldehyde, the compound responsible for the typical flavour of cinnamon, was the main component. The cassia cinnamon kept the same volatile profile after the acidic treatment.

The partial mild acid hydrolysis treatment of cinnamon cassia with citric acid is able to be implemented industrially to provide a decrease of the viscosity while keeping the characteristic aroma profile of cinnamon. Therefore, in food industry is possible to use cinnamon as flavouring and thickening agent using cinnamon cassia, whereas low viscosity products are possible to obtain using a partial acid hydrolysis.

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TILE-BASED ALGORITHM: A FIRST-LINE TOOL TO EVALUATE DIFFERENCES AMONG GRAPE SAMPLES

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Grape composition is known to affect the organoleptic properties of wine and, therefore, its assessment during grape development is essential to ensure the quality of the final product. Techniques based on mass spectrometry are frequently used to evaluate the composition of grapes, however new methods for data analysis are still sought to make its use more intuitive and faster and, if possible, to improve their efficiency. The aim of this work is to assess the applicability of a tile-based algorithm for the discrimination of grape samples with different composition.

Samples of Touriga Nacional variety were collected in two different developmental stages, specifically during berry formation and berry ripening to assure different profiles of grape composition. A metabolomic protocol based in liquid chromatography coupled to mass spectrometry [1] was used to analyse samples. Briefly, samples were extracted with water/methanol/chloroform (20:40:40, v/v/v) and the aqueous-methanolic fraction was analysed. Chromatographic separation was achieved with a reversed phase C18 column in gradient mode. Mass spectrometry analysis was performed in positive and negative ionization mode and data was acquired in scan mode. The resulting data were processed using a tile-based algorithm in Matlab environment and its efficiency was evaluated by comparison with the results obtained in MZmine software.

Preliminary results shown that the algorithm was able to assess the main differences of samples collected at different developmental stages. The algorithm was also able to overcome noise and to detect low frequency/low amount compounds, but further refinement is needed to optimize this process.

The proposed chemometric tool has proved to be suitable to assess the zones of interest in each spectrum and to allow the viable selection of m/z values which contributed the most to the distinctive characteristics of grapes.

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Composition of phenolic compounds and antioxidant capacity in reference material candidate for roasted and ground *Coffea arabica* and *C. Canephora*

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Coffee is the second beverage most consumed worldwide mostly due to its flavor and health benefits. Coffee is well known as source of phenolic compounds, moreover several studies has been demonstrated an association between the intake of these compounds and the protective effect against many diseases. Therefore, it is important to characterize the roasted coffee from *Coffea arabica* and *C. canephora* concerning the nutritional and antioxidant aspects.
In this way, the aim of this study was to evaluate the phenolic compounds and antioxidant capacity from blends with different proportions of *C. arabica* and *C. canephora* in a reference material candidate produced with roasted and ground coffee.
The green coffee beans were standardized for medium roasting (240 °C for 14 minutes) and ground (< 600 µm); then each blend was homogenized for 6 uninterrupted hours by a "Y" homogenizer. Blends were composed by *C. arabica* and *C. canephora*, respectively: 100:0, 95:5, 75:25, 50:50, 25:75, 5:95, 0:100. Total phenolic compounds (TPC) were analyzed by Folin-Ciocalteu method (mg gallic acid equivalent/100g^{-1}) and antioxidant capacity was estimated by using ORAC (µmol Trolox/g) and TEAC (µmol Trolox/g) assays.
It was observed with the increase of *C. Canephora* in the blend provided an increase in the amount of TPC, TEAC and ORAC values, this result corroborates with others where *C. canephora* presented more reducing substances. The results showed 3 groups delimited for phenolic content, namely 0: 100 and 5:95; 25:75 and 50:50; 100: 0, 75:25 and 95: 5. While for both TEAC and ORAC, stands out the groups 5:95 and 25:75 and 75:25 and 95: 5.The high antioxidant capacity can be explained by the considerable amount of TPC. These data indicate that the both species have high content of phenolic compounds and antioxidant capacity, however *C. canephora* showed the highest values. Roasted coffee is an important source to increase the bioactive compounds on a diet.

Table 1. Content of Total phenolic compounds and antioxidant activity of blends of reference material candidate for roasted coffee

C. arabica* : *C. canephora	**TPC (Gallic Acid equivalent mg.100g^{-1})**	**TEAC (µmol Trolox/g)**	**ORAC (µmol Trolox/g)**
100:0	3662^{c}	136.7^{c}	582.9^{a}
95:5	3461.5^{c}	121,78^{d}	491,2^{d}
75:25	3463.7^{c}	128.3^{cd}	508.4^{cd}
50:50	3969.5^{b}	138.7^{c}	514.9^{c}
25:75	4037.1^{b}	151.5^{b}	572,9^{ab}
5:95	4619.5^{a}	160.3^{ab}	558,4^{b}
0:100	4505.7^{a}	170.9^{a}	583.9^{a}

Values with the same letters are not significantly different from each other for Tukey test, level of significance 5%.

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The profile of anthocyanins metabolites in human and ewes after red cabbage intake in the context of protective effects on nerve cells

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Anthocyanins belong to natural pigments with a number of biological properties, including neuroprotective properties. However, consumption of food rich in anthocyanins is not synonymous with their good bioavailability, and thus potentially protective effect on the consumer [1]. Therefore, in order to be able to verify that anthocyanins can have a beneficial effect on the body, it is first necessary to determine if these compounds are absorbed and in what form they are present in the body after ingestion.

The research material were preparations obtained from red cabbage. The study used a ovine model (n = 6) with anthocyanin preparations given as 10 mg cyanidins/kg body weight. Before and at specific time intervals after administration of the preparations (1-10 h), blood from the jugular vein and urine were collected. The obtained sample pre-treatment were carried out by solid-phase extraction (SPE). The concentration of native anthocyanins and their metabolites were analysed using micro-HPLC system (LC200, EKSIGENT, Canada) couples with QTRAP 5500 mass spectrometer (AB SCIEX, Canada).

The basic structure of anthocyanins found in red cabbage was cyanidin-3-diglucoside-5-glucoside, which constituted more than 15% of the total anthocyanin content. The acylated derivatives of this compound accounted for the remaining 85%. The main native anthocyanin found in the blood plasma and urine of ewes after administration of red cabbage was cyanidin 3-diglucoside-5-glucoside, while the primary identified metabolite was the methylated cyanidin triglucoside. The highest concentration of anthocyanins in the blood plasma and urine of ewes after administration of preparation of red cabbage was found in two time intervals, i.e. 1-3h and 6-8h. The main low molecular weight metabolite identified in the body fluids of ewes after the administration of red cabbage was protocatechuic acid.

The obtained results indicate that anthocyanins of red cabbage, similarly to humans [1], are absorbed in the body of ewes and occur in the body fluids of these animals in the form of native forms and methylated and low molecular weight derivatives. The observed similarity allow to use the ovine model in studies of the ability of anthocyanins and their metabolites to penetrate the blood-cerebrospinal fluid barrier and, consequently, help to demonstrate whether anthocyanins have the potential positive effects on nerve cells.

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Seasonal variation of *Ulva rigida* nutritional composition cultivated in a land-based integrated multi-trophic aquaculture system

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Macroalgae are a rich and balanced source of nutrients and bioactive phytochemicals with several health benefits [1, 2] although their specific composition depends on distinct factors, including the algae species, seasonality, location of growth and/or cultivation conditions [3]. The present work aimed to evaluate the chemical variation of the green macroalgae *Ulva rigida* produced in a land-based integrated multi-trophic aquaculture (IMTA) system.

The macroalgae were produced by ALGAplus Lda. under an IMTA system that combines a fish commercial semi-intensive aquaculture with seaweed land-based tank system, in four seasons, using two distinct cultivation flow rates ie high flow rate and low flow rate (corresponding to half of the first). Samples were collected bimonthly each season and analysed for their protein, ash, mineral composition, fat, fatty acid profile and dietary fiber.

Overall, protein and fat content of farmed *Ulva rigida* ranged between 7.6 – 23.2 and 0.1 – 1.3 % DW, respectively, reaching the highest levels during autumn/ winter seasons. In turn, total dietary fiber and ashes showed a contrary seasonal tendency, reaching maximum levels in spring (54.4 – 57.1 % DW and 32.5 – 38.7 % DW, respectively). Notably, the latter was particularly characterized by their richness in potassium, magnesium and iron with a sodium/potassium ratio below 1.9. Moreover, variations in the fat content of *Ulva rigida* were observed throughout the seasons, with concomitant changes on their fatty acids profile, regardless preserving the same major components (palmitic, oleic and α-linolenic fatty acids). Regarding cultivation conditions, protein content in *Ulva rigida* was maximized by setting Fr to high, while the opposite trend was observed for fat, ash and dietary fiber contents.

In conclusion, the biochemical profile of *Ulva rigida* cultivated under a land-based IMTA system may be modulated by setting different cultivation conditions conjugated with seasonal patterns. Moreover, this cultivation system may increase macroalgae profitability on the market and foster new biotechnological applications, e.g. as functional food ingredients.

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Characterization of a commercial dry seaweed (*Palmaria palmata*): Composition, antioxidant and natural pigments content

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Macroalgae or seaweeds are easily found in the most diverse marine habitats. All around the globe and from a very early age, they have been playing an important role in the daily life of the coastal populations (especially in several Asian cultures) as a nutrition source due to their accessibility, diversity, nutritional value and flavour. In the last few years, with the growing awareness to the importance of a healthy lifestyle and a balanced diet, non-traditional consumers started to look for new, natural and rich sources of nutrients, recognizing macroalgae in this context, being its consumption growing exponentially [1].
The red seaweed *Palmaria palmata* (also known as Dulse or Dillisk) is abundant in the Atlantic waters and, therefore, is one of the more popular seaweed for human consumption in Europe. Due to its delicate flavour, Dulse is one of the most suitable algae for tasting raw or dried as a snack or incorporated into bread and soups [2]. Apart from their nutritional value, red seaweeds have been reported as a valuable source of bioactive compounds such as phenolics and/or natural pigments with health benefit effects (anti-diabetic, antioxidant, anti-inflammatory, neuroprotective effect) [3]. However, the chemical composition of seaweeds varies depending on their geographical region, harvest season, seasonal changes as well as the processing method to which they are subjected. Due to the high moisture content of fresh material (85-90%), in order to be suitable for commercialization, algae have been traditionally subjected to drying processes. Recently, the algae processing industries are using dry greenhouses with air ventilation during defined periods (48 h) of temperature (42ºC), before being placed on the food market in bags.
The aim of the present work was to characterise the *Palmaria palmata* from the Atlantic coastal region of Galicia, commercially available in industrial dry form. Nutritional analysis were performed using AOAC methods [4]. Antioxidant activity was accessed in several types of extracts (obtained using different solvents and times of extraction). The content of non-polar (chlorophylls and carotenoids) and polar (phycobiliproteins) pigments were also evaluated by spectrophotometric assays using methanol and phosphate buffer, respectively, as solvent.

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Improving the extraction and isolation of proteins from *Porphyra* spp.

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Seaweed, especially red species (Rhodophyta), are known to have a high protein content, this makes them a potentially rich source of bioactive peptides [1]. This study focused on improving the extraction and isolation of proteins from two red seaweed species - *Porphyra dioica* and *Porphyra umbilicalis*.

Seaweed samples were produced in an integrated multitrophic aquaculture system, dried (25 ºC, moisture content <10%) and milled prior to analysis. Protein extraction was performed using two protocols: i) an aqueous followed by an alkaline extraction (P1) and ii) a direct alkaline extraction (P2). In P1, the water soluble proteins were firstly extracted with deionised water and were collected by centrifugation. The pellet remaining was extracted twice with NaOH, and both the aqueous and the alkaline supernatants were combined for further analysis. In the P2 process, the proteins were extracted directly in NaOH. The protein in all extracts was harvested by isoelectric precipitation (IP) at different pH values (pH 3.0, 3.5, 4.0 and 4.5). The protein content in the precipitates was estimated using a modified Lowry method [2, 3].

The total protein yield for *P. umbilicalis* was higher using protocol P1 compared to P2 (71.22 *vs* 64.79 mg protein/g sample, dw, respectively). In the case of *P. dioica*, P2 gave a higher total protein yield compared to P1 (73.92 *vs* 43.22 mg protein/g sample, dw, respectively). IP at pH 4.0 was more efficient for *P. umbilicalis* (61.05±0.75 mg protein/g sample, dw) compared to IP at pH 4.5, 3.0 and 3.5 (55.41±4.92, 36.27±5.08 and 36.25±8.04 mg protein/g sample, dw, respectively). Whereas, for *P. dioica* IP at pH 4.5 was found to be more effective (42.2±0.50 mg protein/g sample, dw) compared to pH 3.5, 4.0 and 3.0 (32.14±1.22, 31.36±1.48 and 29.74±0.78 mg protein/g sample, dw, respectively).

In summary, protein extraction and IP protocols were established: for *P. umbilicalis*, protocol P1 (aqueous followed by alkaline extraction) followed by IP at pH 4.0, and for *P. dioica* protocol P2 (aqueous-alkaline combined extraction) followed by IP at pH 4.5 gave optimum yield. Taking into consideration the complexity of seaweed, the results of this study demonstrate the importance of optimizing the protein extraction and isolation protocols for each species, especially when the final goal is to obtain the highest protein yield from each sample.

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Phenolic compounds extraction from Algerian *Salvia chudaei* Batt & Trab.

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Medicinal and aromatic plants have been traditionally used to treat common disfunctions. Their therapeutic properties can be due to their metabolites (such as phenolic compounds) and synergies between them. Medicinal and aromatic plants are widely used in Algeria, especially in the isolated areas (*e.g.* Hoggar in the South of Algeria) where the conventional medicine is often not available [1]. *Salvia chudaei* Batt. & Trab. is a medicinal plant belonging to Lamiaceae family, endemic of Central Sahara. It was first observed in the Tamanrasset region (south of Algeria) in 1905. The local people use it to treat dysmenorrhea, abdominal pain, spasms, rheumatism, digestive and renal diseases, urinary retention, urinary tract infection and prostate pain [2,3,4]. Scientific studies carried on *Salvia* sp., especially wild ones, report high phenolic contents with interesting bioactivities and health promotion properties such as anti-inflammatory [5], cytotoxic, antioxidant, anti-Alzheimer [6] and anticancer [7].

The aim of this work was to optimize the phenolic compounds extraction from the aerial parts of *S. chudaei* Batt. & Trab. Different extraction solvents and sample weights were tested ethanol-water (50:50 and 80:20) and methanol-water (80:20). The temperature of extraction and solvent volumes were also controlled. Figure 1 shows the total phenolics content (TPC) of extracts using the different extraction conditions.

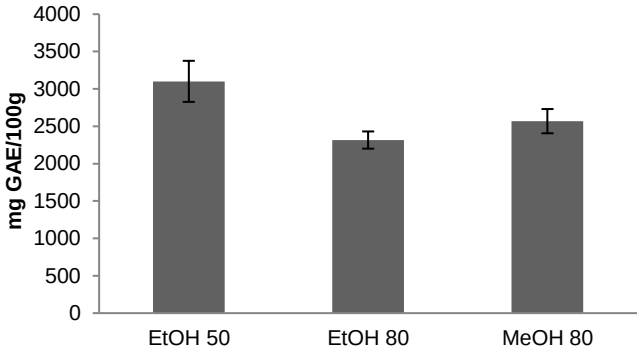

Fig.1. TPC of different extraction solvents

The results have shown that ethanol-water (50:50) was the most suitable for the phenolic compounds extraction, and the 0.2 % solid-liquid ratio seems to give the highest recovery. Phenolics profile was analysed by reverse phase HPLC-DAD. Rosmarinic acid (RA) was the major phenolic identified in the sample.

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Real-Time Authentication of food and beverages using DART-QDa LiveID Analysis

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Currently there is no globally harmonised definition for "food fraud" however, there are initiatives coordinating action against fraudulent practices in the food supply chain. Food and beverages of higher commercial value are more frequently subject to fraudulent practice. Whiskey is sold as the product of one distillery or as a blend. Analytical methods are required for process control, quality assurance and formulation. Whiskey characteristics are influenced by the cereals used in fermentation, distillation, maturation and blending regimes. Palm oil is also a commodity of socioeconomic importance. Analysis of the characteristic profiles of such products can be conducted using gas chromatography for authenticity purposes. Such methods are time-consuming and there is a need for rapid, qualitative testing to keep pace with industry requirements.

Direct Analysis in Real Time (DART) is an ambient ionization technique with minimal sample preparation and no chromatography. DART was coupled to a single quadruope mass spectrometer to produce spectral profile data. Linear X rail module was used for sample introduction purposes utilizing the QuickStrip cards. The DART source was operated with a helium gas flow of 1.5 L/min heated to 400oC. The MS was operated in full scan mode across mass range m/z 150-950 in both positive and negative polarity modes with a cone voltage 5-30 V with a scanning rate of 2 Hz. Multivariate statistical models were generated using authentic characterized samples; subsequently the models were used for real-time classification of unknown whiskey and palm oil samples.

Within this study we have investigated the potential for the implementation of DART QDa LiveID as solution for the rapid authentication of high value food and beverage samples of both liquid and solid types with minimal sample preparation. Proof of concept multivariate models for product authenticity have been generated using a collection of authentic reference samples of single malt whiskey (brand 1, brand 2 and adulterated sample) and palm oils including crude, sustainably produced crude, refined originating from different geographical locations.

DART produces relatively simple mass spectra characterized by M+. , [M+H]+ in positive mode and M-. , [M+H]- in negative mode. Fragment ions are observed for some compounds, the degree of fragmentation can be influenced by the choice of gas. In both the whiskey and palm oil applications, positive polarity ionization was found to give most abundant spectral features and considered to be the more diagnostic mode for model training. In the case of the whiskey, ions with m/z between 50–300 we seen to be responsible for the brand level differences whereas ions at between m/z 300-400 drove the differences between the authentic and adulterated samples. In the palm oil, ionized species suspected to be generated from saturated fatty acids (medium-long chain); carotenoids (phytoene, α-cartone, β-cartonene, lycopene); vitamin A (retinol, retinal, retinoic acid); tocopherols (α,β,γ,δ); tocotrienols (α,β,γ,δ); diglycerides and triglycerides were evident in the full scan spectral data. On the basis of these spectral features, it has been possible to generate a multivariate model using a combination of Principal Component Analysis (PCA) for data dimension reduction and Linear discriminate analysis (LDA) for class discrimination to distinguish between grades of palm oil processing and production type.

Fourier-transform near infrared spectroscopy as a tool to predict the composition of olive pomace blends

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Olive oil production generates large amounts of olive pomace (OP), which is an environmental problem due to its high content in phytotoxic compounds, as phenolics. However, such compounds have health-beneficial properties (*i.e.* antioxidant) and, thus, their recovery is of high interest for *e.g.* food industry. Antioxidant activity can be easily measured with classic colorimetric methods. However, they are time-consuming, relatively expensive and less eco-friendly when adapted to industrial scale for routine analyses [1,2]. Industries need to use faster and low-cost methods for raw-material assessment. The on-line tests during manufacturing can play a key role on the assessment of antioxidants content. Fourier-transform near infrared spectroscopy (FT-NIR) is a green analytical technique that provides information about several chemical properties in a single measurement.

In this work 53 samples were prepared from blends of four monovarietal OP (Arbosana, Oliana, Arbequina and Koroneiki) from Alentejo, Portugal. An experimental design was used to achieve different composition percentages of the individuals OP. The samples were analysed through FT-NIR spectroscopy [1] and chemical methods, namely total phenolics content (TPC) and antioxidant activity [3]. This work aimed to correlate the chemical analysis with FT-NIR spectra to distinguish OP samples (monovarietal and blends) with a higher amount of bioactive compounds.

The TPC and antioxidant capacity determination of OP samples using FT-NIR spectra and partial least squares (PLS) regression yielded poor results in terms of determination coefficients. This could be attributed to the reduced range of all the parameters evaluated. Nevertheless, the determination of the percentage of each OP variety in all the blends yielded good results with determination coefficients higher than 0.9 for all the varieties.

The chemical analysis showed that the proportion (%) of Arbosana, Koroneiki, Oliana, and Arbequina 2.75:65:29:3.25, respectivelly, was the one with higher amount of TP (21.22 g gallic acid equivalents (GAE)) whereas the sample with the proportion (3.5:85:2.75:8.75) presented the highest antioxidant activity by FRAP (59.72 g ferrous sulfate equivalents) and the proportion 1.5:90:4.75:3.75 presented the higher scanvenging activity (21.53 g equivalents trolox). Individual OP Koroneiki is the sample with higher amount of phytochemicals (15.39 g GAE/kg of OP) which correlates with the samples with higher proportion of this OP.
Although the FT-NIR spectroscopy was not able to accurately determine TPC and antioxidant activity it was possible to characterize OP blends in terms of its composition. In this way, by knowing which OP varieties presents a higher TPC content and antioxidant activity, it is possible to indirectly characterize its bioactive properties.

Overall, the results obtained demonstrated that FT-NIR spectroscopy could be an interesting, quick and environmentally friendly technique to distinguish the composition of OP samples. This technique will allow selecting the OP samples that have a higher amount of antioxidants, namely phenolics, for further extraction procedures. In this way, this method can be an effective valorisation tool for OP.

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POSTERS

Functional Foods

Phenolic content, starch and fibre on gluten-free pasta elaborated with rice, bean and carob fruit. Effect of cooking processing.

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In general, gluten free (GF) pasta are considered to have inferior quality than traditional due to the lack of gluten characteristics. In GF pasta, the use of a traditional source of starch (TS), such as rice fortified with legumes is one option in order to assume the structural role of gluten, being of great interest the amylose/amylopectin ratio of the starch utilized [1]. Resistant starch (RS) consumption is associated with several health benefits: enhanced vitamin and mineral absorptions; lower triglyceride concentrations and plasma cholesterol; and prevention of colon cancer [2]. Nowadays, dietary fibre has been related to lowering risk colorectal cancer, diabetes, constipation or cardiovascular diseases [3], however its consumption is below the daily recommended intake (25 g/day). Phenolic compounds are considered as natural antioxidants, they are preventive agents against several degenerative diseases, such as cancer and cardiovascular diseases [4].

The aim of this work was to determine the total and resistant starch, amylose and amylopectin content, dietary fibre fractions and different phenolic compounds on pasta elaborated with 0-100% rice (*Oryza sativa*), 0-100% bean (*Phaseolus vulgaris*) and 0-10% carob fruit (*Ceratonia siliqua*). At the same time and taking into account that pasta is consumed after cooking in water and this process can induce great changes in the content of different compounds, this study analysed also the cooked samples to assess the real amount of the studied compounds that are ingested by the consumers. Moreover, the results were compared with a commercial rice pasta easily available in the market, allowing people to make informed choices.

The presence of legumes in the formulations reduced the amount of TS detected. Thus, TS content of the different uncooked fettuccine was around 35%-60%. The cooking process increases slightly the starch content, although there were not significant ($p>0.05$) differences between uncooked and cooked samples. The higher legumes amount in the formulation, the higher RS, amylopectin, dietary fibre fractions and phenolic compounds content were detected. The main dietary fibre fraction detected corresponded to insoluble fibre. The pasta formulated with 10% carob fruit showed a higher ($p<0.05$) fibre and phenolic compounds content than their corresponding counterpart without this legume. Cooking process produced significant changes (in almost formulations) on RS, dietary fibre and total phenols content. While a significant ($p<0.05$) reduction of RS, amylose and amylopectin was observed, the dietary fibre slightly increases, being, mainly, affected the soluble fraction. The different phenolic groups analysed (total phenols, tartaric esters, flavonols and anthocyanins) were reduced (17-48%) after cooking. These decreases can be attributed to the lixiviation of these compounds to the cooking water during fettuccine processing, which would change the relative composition of the experimental samples. In comparison to the commercial rice/corn pasta, all the samples (uncooked and cooked) showed higher amount of RS, dietary fibre and phenolic compounds.

We can conclude that the different compounds studied were not affect in the same extent during the pasta elaboration and the cooking process. Taking into account the composition of uncooked and cooked studied samples, all the fettuccine would be considered as a healthier alternative to the commercial/external control.

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Biorefining strawberry pomace into functional ingredients using multistep High Pressure Extraction methods

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Effective processing of fruit pomace may generate valuable functional ingredients for developing food products with higher amounts of different dietary polyphenols and other health beneficial nutrients. In addition, biorefining of agro-food by-products ensures more effective their management targeting a final 'zero waste' concept. The aim of this work was to recover valuable substances from strawberry pomace (*Fragaria x ananassa)* by different extraction methods and to evaluate antioxidant properties, composition of phenolic and aroma compounds of obtained products.

Extracts were obtained using conventional and more advanced extraction methods, such as pressurized liquid extraction (PLE), supercritical carbon dioxide (SC-CO_2) extraction both applied separately and consecutively (as well as their combinations SC-CO_2 residue macerated with ethanol (EtOH); EtOH residue macerated with water (H_2O). SC-CO_2) extraction parameters (pressure, temperature and dynamic extraction time) for recovery of lipids were optimized by using Response Surface Methodology with Central Composite Design. The effectiveness of the extraction was evaluated by measuring the changes in antioxidant properties of solid plant material before and after extraction. For this purpose QUENCHER procedure (direct antioxidant capacity evaluation in a solid matrix) using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging, oxygen radical absorbance capacity (ORAC) of total phenolic content assays was used. Triacylglycerol's, anthocyanins, flavonoids, organic acids and fatty acids in the extracts obtained were determined by ultra-performance liquid chromatography – quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) and gas chromatography with a flame ionization detector (GC-FID), respectively. The composition of volatiles of strawberry SCFE-CO_2 extracts was analysed by gas chromatography time-of–flight mass spectrometry (GC-TOF/MS).

The highest extraction yield (7.65 g/100g dry weight) was obtained using 36 MPa pressure, 68°C temperature and 154 min dynamic extraction time. The main fatty acids in SC-CO_2 extracts were linolenic (Ln), linoleic (L), stearic acid and oleic (O), while the main identified triacylglycerols were LnLnLn, LLnLn, LLLn, LnLnLn, LLL, OLL, OOL, OOO. Evaluation of antioxidant properties showed that after various extraction processes the residues still contained remarkable radical scavenging capacity and total phenolic content. The main anthocyanin glycosides identified in strawberry pomace were cyanidin-3-glucoside, pelargonidin-3- glucoside, pelargonidin-3-rutinoside, pelargonidin 3- (6''-malonyl-glucoside), delphinidin-3-glucoside. The main organic acids and other phenolics were malic, citric, succinic, kaempferol, quercetin and their glucosides and glucopyranosides, ellagic acid, p-coumaric acidS and their glucosides. The main volatiles in SC-CO_2 extracts were γ-decalactone (9.3%), γ-dodecalactone (7.5%), ethyl hexadecanoate (8.9%), methyl linoleate (6.3%), nerolidol (5.9%), nonacosane (5.0%), squalene (2.5%), 2E,4E-decadienal (2.7%).

In general, strawberry pomace is a good source of dietary antioxidans, and may be a promising functional ingredient for increasing nutritive value of foods and development of nutraceuticals.

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The mechanisms of hypoglycemic effect of foxtail millet based on transcriptomics

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Our previous animal experiment has proved that foxtail millet feeding significantly improved the blood glucose metabolism in high fat diet and STZ-induced (HFD/STZ) diabetic rats. To further clarify the mechanisms of the hypoglycemic effect of foxtail millet, the difference on liver transcriptional profiles between diabetic rats, normal rats and foxtail millet feeding rats were investigated. The results shown that 4 weeks of foxtail millet feeding in this study could mitigate negative variations of liver transcriptional profile in HFD/STZ diabetic rats. Specifically, foxtail millet feeding activated the insulin-stimulated PI3K/AKT signaling pathway by up-regulating expression of IRS, PI3K and AKT in liver, which will inhibit gluconeogenesis by down-regulating expression of G6P, FBP and PEPCK, and stimulate glycolysis by up-regulating expression of GK and PK subsequently. Moreover, foxtail millet feeding inhibited NF-κB signaling pathway and reduced expression of inflammatory factors, which will weaken the inhibition of insulin signaling pathway and improve blood glucose metabolism ultimately.

Impact of in vitro gastrointestinal digestion on phenolic compounds and antioxidant activity of different coffee blends

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Coffee is one of the most consumed beverages and has been linked to health in various research studies. Several studies have indicated that a strong antioxidant activity of coffee is associated with it is content of phenolic compounds as well as Maillard reaction products, the latter being generated during roasting[1]. Chlorogenic Acids (CGAs), which include many different isomeric forms, are the predominant phenolic compounds in coffee beans. Single-dose coffee capsule system is a recent technology used to prepare espresso coffee quickly and which offers consumers the possibility to choose among several blends and, normally this blend is a mixture of Coffea arabica (Arabica) and Coffea canephora (Robusta). In vitro gastrointestinal digestion (GID) models based on human physiology are important tools in the assessment of phenolic compounds during GID, particularly because of their simplicity, low cost and bio-accessibility of nutrients. The aim of this study was to evaluate the impact of in vitro gastrointestinal digestion on phenolic compounds and antioxidant activity of the different coffee blends extracted with a single-dose capsule system, for a medium roasting.
Three different coffee blends supplied by Delta Cafés Company were submitted to an in vitro gastrointestinal digestion[2]. In order to monitor the phenolic content and antioxidant activity, samples of coffee were collected from different stages of digestion: mouth, gastric and intestinal digest. The total phenolic content (TPC) was analysed by using the Folin-Ciocalteu method[2]; the antioxidant activity was evaluated by using ABTS˚ and ORAC method[2]. The phenolic compounds profile was determined by HPLC[2].
The phenolic profile of regular and digested coffee was the similar but exhibiting different concentrations. The main compounds were: neochlorogenic acid (NGA), chlorogenic acid (CGA), cryptochlorogenic acid (CCGA), 4,5-Dicaffeoylquinic acid (4,5-Di-O-CQA), 3,5-Dicaffeoylquinic acid (3,5-Di-O-CQA) and 3,4-Dicaffeoylquinic acid (3,4-Di-O-CQA). In all coffee blends after GID, the content of phenolic compounds (total and individual) decreased as well as antioxidant activity. Chlorogenic acid was, in all coffee blends, the phenolic compound most degraded by the GID, presenting losses of approximately 70%.

Table 1. Concentration of chlorogenic acids in mg/coffee (30 mL) at the different stages of digestion.

Coffee Blend	Stage of digestion	NGA	CGA	CCGA	4,5-Di-O-CQA	3,5-Di-O-CQA	3,4-Di-O-CQA
6	T0	$17,0^{a} \pm 0,5$	$29,7^{a} \pm 0,2$	$16,9^{a} \pm 0,3$	n.d	n.d	n.d
	Mouth	$15,0^{ab} \pm 0,2$	$25,1^{ab} \pm 0,2$	$14,5^{bc} \pm 0,2$	n.d	n.d	n.d
	Gastric	$12,0^{bc} \pm 0,1$	$19,4^{bc} \pm 0,5$	$15,4^{ab} \pm 0,1$	n.d	n.d	n.d
	Intestinal	$9,5^{c} \pm 0,1$	$8,1^{c} \pm 1,2$	$9,5^{c} \pm 0,9$	n.d	n.d	n.d
8	T0	$17,0^{a} \pm 1,8$	$32,1^{a} \pm 3,6$	$17,1^{a} \pm 1,8$	$7,7^{a} \pm 1,1$	$1,2^{c} \pm 0,1$	$2,2^{b} \pm 0,3$
	mouth	$14,7^{b} \pm 0,7$	$27,0^{b} \pm 1,6$	$15,0^{b} \pm 0,8$	$5,3^{b} \pm 0,4$	$0,9^{d} \pm 0,1$	$1,4^{c} \pm 0,1$
	gastric	$11,5^{c} \pm 0,4$	$19,8^{c} \pm 0,7$	$15,4^{ab} \pm 0,5$	$4,7^{c} \pm 0,1$	$3,1^{a} \pm 0,0$	$3,3^{a} \pm 0,0$
	intestinal	$11,0^{c} \pm 0,3$	$11,3^{d} \pm 0,5$	$11,5^{c} \pm 0,4$	$2,9^{d} \pm 0,1$	$2,5^{b} \pm 0,0$	$2,6^{b} \pm 0,0$
12	T0	$16,1^{a} \pm 1,2$	$26,4^{a} \pm 1,0$	$15,1^{a} \pm 1,7$	$6,4^{a} \pm 1,3$	$1,1^{c} \pm 0,2$	$2,0^{c} \pm 0,3$
	mouth	$13,1^{b} \pm 0,3$	$23,0^{b} \pm 0,2$	$13,6^{b} \pm 0,2$	$5,0^{a} \pm 0,1$	$0,8^{d} \pm 0,0$	$1,2^{d} \pm 0,0$
	gastric	$9,6^{c} \pm 0,3$	$15,7^{c} \pm 0,5$	$13,1^{c} \pm 0,4$	$4,5^{b} \pm 0,1$	$3,1^{a} \pm 0,0$	$3,2^{a} \pm 0,0$
	intestinal	$10,2^{d} \pm 0,2$	$9,7^{d} \pm 0,3$	$11,0^{d} \pm 0,3$	$2,8^{c} \pm 0,0$	$2,7^{b} \pm 0,0$	$2,6^{b} \pm 0,0$

*n.d: not detectable.

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Identification of a novel anticancer oligopeptide from *Perilla frutescens* (L.) Britt. and its enhanced anticancer effect by targeted nanoparticles in vitro

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Perilla frutescens (L.) Brittis is a dietary herbal medicine and has anticancer effect. However, little is known about its anticancer peptides. This study is aimed at identifying cytotoxic oligopeptides which are loaded by a drug delivery system, to explore its anticancer application. The oligopeptides were isolated from enzymatic hydrolysates of Perilla seed crude protein by using ultrafiltration, gel filtration chromatography, and reversed-phase high-performance liquid chromatography (RP-HPLC). The structure of the oligopeptide was determined using a peptide sequencer, and its anticancer effect was examined by the MTT assay. PSO (Perilla seed oligopeptide), the most potent anticancer oligopeptide, was loaded by chitosan nanoparticles (NPs) modified by hyaluronic acid (HA). Then, the particle size, zeta potential, encapsulation effciency (EE), drug loading effciency (LE), the cumulative release rates of NPs, and its cytotoxic effect on cancer cells were investigated. Three fractions were isolated by the chromatography assay. The third fraction has a broad-spectrum and the strongest anticancer effect. This fraction was further purified and identified as SGPVGLW with a molecular weight of 715Da and named as PSO. Then, PSO was loaded by HA-conjugated chitosan to prepare HA/PSO/C NPs, which had a uniform size of 216.7nm, a zeta potential of 35.4mV, an EE of 38.7%, and an LE of 24.3%. HA/PSO/C NPs had a slow release rate in vitro, with cumulative release reaching to 81.1%. Compared with free PSO, HA/PSO/C NPs showed notably enhanced cytotoxicity and had the strongest potency to human glioma cell line U251. This study demonstrated that PSO, a novel oligopeptide from Perilla seeds, has a broad-spectrum anticancer effect and could be encapsulated by NPs, which enhanced tumor targeting cytotoxicity with obvious controlled release. Our study indicates that Perilla seeds are valuable for anticancer peptide development.

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Nutritional and phytochemical properties of *Opuntia ficus-indica* (L.) Miller fruits from Portugal

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Opuntia ficus-indica (L.), commonly referred to a cactus pear, belongs to the Cactaceae family, characterized by a remarkable adaptation to arid and semi-arid climates, in tropical and subtropical regions of the globe. In Portugal, it is known as "figueira do diabo".The fruit can be used in the production of jams, in cosmetic applications and folk medicine, based on its bioactive compounds and biological/pharmacological properties [1].

The aim of this study was to compare the proximate composition of three fruit varieties (red, yellow and green) of *O. ficus-indica* provided by a local producer in Torres Novas, Portugal. Nutritional parameters were determined using AOAC official methods [2]. In addition, total phenolics and total flavonoids contents, DPPH• inhibition and the ferric reducing antioxidant power (FRAP) of the samples were also assessed by spectrophotometric procedures [3]. Antioxidants extraction was performed using different solvents: (a) 100% water, (b) water/ethanol (1:1), and (c) 100% ethanol.

The nutritional profile of the three fruit varieties is presented in Table 1. In general, these fruits are poor in fat and a source of dietary fiber, especially insoluble one. Although the proximate composition is similar for all the samples, the red fruits presented a slightly higher content of protein. In turn, yellow fruits contained the highest levels of total minerals.
Regarding the antioxidant activity, the mixture water/ethanol (1:1) allowed the most efficient extraction. The red fruits showed the highest antioxidant activity among all the samples, while the green ones had the lowest score.

Table 1. Proximate composition of *Opuntia ficus-indica* fruit varieties.

Sample	Moisture (%)	Ash*	Fat*	Protein*	Total fiber*	Insoluble fiber*	Soluble fiber*	Carbohydrates*
Red	80.53±1.22	3.86±0.06	1.50±0.09	6.40±0.41	27.27±0.36	19.96±0.30	7.80±0.60	60.90 ± 0.16
Yellow	80.69±0.99	4.19±0.07	1.30±0.10	4.09±0.28	26.76±0.05	18.10±0.50	8.60±0.50	62.62 ± 0.10
Green	79.79±1.16	3.96±0.07	1.20±0.06	5.33±0.27	29.64±0.90	20.90±0.20	8.70±0.08	60.16 ± 0.03

Results are presented as mean ± standard deviation of replicates(n=3);*expressed in g/ 100g dw

The results of the different fruit varieties can be useful to better understanding the differences among them and help consumer to make a cleared purchase decision. This study also aims to improve the knowledge and the demand of these fruits by the Portuguese population.

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The Glucose-Lowering Effect of Foxtail Millet in Subjects with Impaired Glucose Tolerance: A Self-Controlled Clinical Trial

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Foxtail millet has relatively low starch digestibility and moderate glycemic index compared to other grains[1, 2]. Since there are still no clinical researches regarding its long-term effect on blood glucose, this self-controlled study was conducted to investigate the glucose-lowering effect of foxtail millet in free-living subjects with impaired glucose tolerance (IGT). Fifty g/day of foxtail millet was provided to enrolled subjects throughout 12 weeks and the related clinical parameters were investigated at week 0, 6 and 12, respectively. After 12 weeks of foxtail millet intervention, the mean fasting blood glucose of the subjects decreased from 5.7 ± 0.9 mmol/L to 5.3 ± 0.7 mmol/L ($p < 0.001$) and the mean 2 h-glucose decreased from 10.2 ± 2.6 mmol/L to 9.4 ± 2.3 mmol/L (p = 0.003). The intake of foxtail millet caused a significant increase of serum leptin ($p = 0.012$), decrease of insulin resistance ($p = 0.007$), and marginal reduction of inflammation. Furthermore, a sex-dependent difference in glucose-lowering effect of foxtail millet was observed in this study. Foxtail millet could improve the glycemic control in free-living subjects with IGT, suggesting that increasing the consumption of foxtail millet might be beneficial to individuals suffering from type 2 diabetes mellitus.

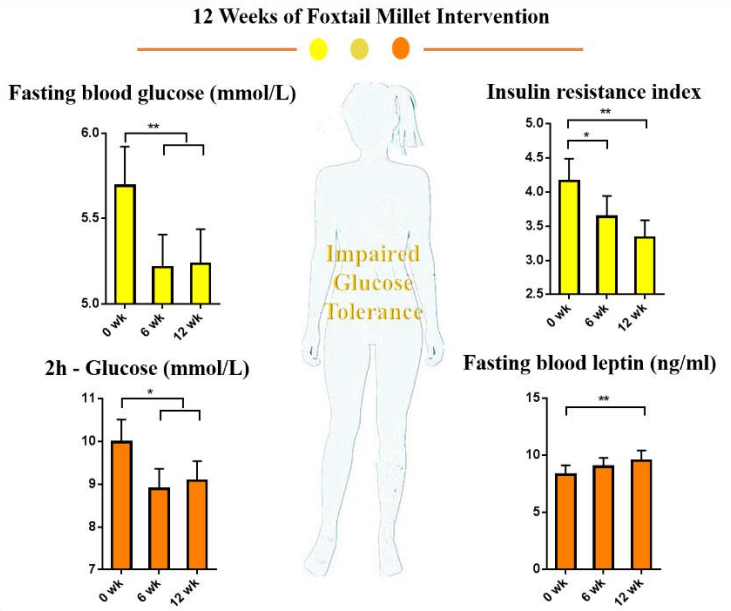

Fig.1. Graphical Abstract

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Evaluation of the phenolic compounds and antioxidant activity of nine medicinal plant materials

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The medicinal plants, occupying a great position both in agricultural and industrial productions are particularly regarded as rich sources of plant-based nutritional supplements, medicinal drugs and active substances, largely due to their phenolic composition. These phenolic compounds are of great interest because of numerous biological activities and benefits; among them the antioxidant activity or free radical scavenging capacity is the most fundamental and principal which is responsible for resisting oxidative stress in human body. In addition, leaves were regarded as the most utilized plant parts for the preparation of ethno-medicines, likely due to the presence of active compounds and comparative ease of phytochemical and pharmacological studies compared to other parts.

In order to find out new sources of safe, inexpensive and functional formulations, phytochemical contents of nine medicinal plant materials (leaf of *Salvia officinalis*, *Rosmarinus officinalis* and *Olea europaea*, aerial part of *Ruta graveolens*, *Mentha piperita*, *Petroselinum crispum* and *Tribulus terrestris*, leaf and flower of *Punica granatum*), screened and collected in Botanic Garden (UTAD), were determined with hydro-methanolic extraction and colorimetric analysis, as well as their antioxidant activity *in vitro* were assessed by ABTS, DPPH and FRAP spectrophotometric assays.

It could be concluded that the contents of total phenols and *ortho*-diphenols in *Punica granatum* material extracts were significantly higher than that in the other plant materials, with a wide variation of about 29 and 15 folds, respectively. Furthermore, these plant exracts from *Punica granatum* also showed obvious strengths of antioxidant activity, compared with others in this study. Therefore, the leaf and flower of *Punica granatum* would be important sources of bioactive phenolics and have the potential to be developed into health-promoting products in food and pharmaceutical industries.

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Quantification of L-theanine on different parts of Azorean *Camellia sinensis* in two plantation zones under different drying temperatures

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Tea plant, originally from China, gradually expanded into many tropical countries, and since the last decade of the 19th century is also produced in one unique place in Europe - S. Miguel, Azores Archipelago [1]. *Camellia sinensis* tea has received considerable attention due to its scientifically proven beneficial effects on health [2]. These effects have been attributed to the non-protein amino acid (AA) L-theanine, the predominant AA in *C. sinensis* that can maintain normal sleep, improve memory function and has influential effects on lifestyle associated diseases, such as: stress relief, cardiovascular disorders, diabetes, hypertension, tumor suppression and liver injury [3].The objective of this study was to quantify L-theanine, using samples from two different plantation zones and different drying methods in order to investigate the influence of drying process on the L-theanine stability. The samples were dried at 70 ºC and 120 ºC (processed in tea factory) for green and black tea samples, respectively, and dried at 45 ºC for green tea samples (processed in laboratory for a longer time). The results presented in Table 1 revealed that temperature strongly affect the level of tea L-theanine content. The highest levels of L-theanine were observed in internodes for all samples, as compared with leaves samples, especially for green tea processed in laboratory that showed the values of 20.26 mg/g and 26.91 mg/g of DW for A and B zones, respectively, as compared with samples processed in tea factory. The L-theanine content in the internodes processed in the tea factory showed higher values of 17.48 to 17.98 mg/g of DW for green tea as compared to 9.51 to 6.59 mg/g of DW for black tea in zones A and B, respectively, however, that differences is must lower in the leaves samples, that ranged from 5.82 to 6.51 mg/g of DW for green tea as compared to 5.43 to 5.59 mg/g of DW for black tea. The highest values were also observed in green tea leaves processed in laboratory (8.22 mg/g and 12.02 mg/g of DW for A and B zones, respectively).

In conclusion, temperature influence the levels of L-theanine in both green and black tea samples, showing strong evidence in the green tea processed in the laboratory using longer drying times and lower temperatures. The highest levels in Internodes as compared to Leaves can be explained by the fact that L-theanine is used as the skeleton for the leaves catechins formation under the influence of sunlight exposure. The difference in the L-theanine content in different tea plantation zones may be explained by different soil composition, that interfere with the L-theanine synthesis in the root of plant and/or different sunlight exposure during the growing process.

Table 1. L-Theanine contents on different parts of *C. sinensis* under different drying temperatures[a]

***Camellia sinensis* parts**	**L-Theanine in mg/g of dry weight (DW)**					
	Processed in Tea Factory				Processed in Laboratory	
	A		B		A	B
	Green Tea	Black Tea	Green Tea	Black Tea	Green tea	
Leaves	6.51 ± 0.09	5.43 ± 0.42	5.82 ± 0.05	5.59 ± 0.06	8.22 ± 0.02	12.02 ± 0.09
Internodes	17.48 ± 0.38	9.51 ± 0.24	17.98 ± 0.66	6.59 ± 0.04	20.26 ± 0.26	26.91 ± 1.47
Leaves+Internodes	11.70 ± 0.13	8.11 ± 0.14	12.12 ± 0.27	6.19 ± 0.09	13.70 ± 0.32	19.73 ± 0.35

[a]Values are mean ± SD (*n = 3*). A (Lat 37.818 and Long -25.404) and B (Lat 37.816 and Long -25.401) zones

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Determination of total phenolics, total flavonoids and antioxidant properties of different parts of Azorean *Camellia sinensis*

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Originated from China, the tea plant (*Camellia sinensis*) gradually expanded into many tropical countries and since the last decade of the 19th century, tea is also produced in one unique place in Europe: S. Miguel Island, Azores [1]. *C. sinensis* tea has received considerable attention for its source of polyphenols, the major antioxidant constituents of tea that are responsible not only for their flavor characteristics, but also for their wide variety of health benefits [2]. This study explored the biological activities of different parts of Azorean *C. sinensis* extracts from green tea in order to find the best blend that maximize its effect on health.

The results (Table 1) show that Bud presented higher and similar EC_{50} values of FRSA and FRAP (6.10 µg/mL and 5.70 µg/mL, respectively). Combination of Bud, 1st plus 2nd leaf and Internodes shows the highest FIC activity value (79.21%) followed by the combination of Bud, 1st plus 2nd leaf (78.35%) and Bud (74.88%). According to Chan *et al.* [3], young leaves present high EC_{50} values (30 µg/mL) for FRSA, less antioxidant activity than the results observed in our study. The internodes show lower values of FRSA, FRAP and FIC (13.50 µg/mL, 11.40 µg/mL and 36.96%, respectively). The TPC results are very similar for all *C. sinensis* parts with a high content in 2nd leaf (265.48 mg GAE/g DE) and a lower value for Internodes (201.51 mg GAE/g DE) that is higher than the results reported by Nibir *et al.* [4]. The 2nd leaf presented also a high TFC content of 72.02 mg RE/g DE, followed by the combination of Bud, 1st plus 2nd leaf and Internodes (54.06 mg RE/g DE) and lower content in Internodes (23.84 mg RE/g DE). The different parts of Azorean *C. sinensis* extracts from green tea have significant antioxidant activities and high TPC and TFC. In conclusion, drinking green tea may have health benefits and also shows to be a potential functional food or an added-value food ingredient.

Table 1. Free radical scavenging activity (FRSA), Ferric reducing antioxidant power (FRAP), Ferrous Ion-Chelating (FIC) activity, Total Phenolics (TPC) and Total Flavonoids (TFC) contents in dry sample extracts of different parts of *Camellia sinensis*[a]

Different parts of *Camellia sinensis*	FRSA (EC_{50}[b], µg/mL)	FRAP (EC_{50}[c], µg/mL)	FIC (%)	TPC (mg GAE/g DE)	TFC (mg RE/g DE)
B	6.10 ± 0.33	5.70 ± 0.10	74.88 ± 1.53	253.23 ± 3.08	29.52 ± 0.92
I	13.50 ± 0.57	11.40 ± 0.10	36.96 ± 3.23	201.51 ± 3.36	23.84 ± 1.25
1st L	10.50 ± 0.70	9.40 ± 0.15	55.94 ± 2.33	254.25 ± 3.33	53.61 ± 0.47
2nd L	10.70 ± 0.64	8.50 ± 0.09	49.34 ± 0.59	265.48 ± 2.06	72.02 ± 1.33
B+1st+2nd L	9.00 ± 0.89	9.60 ± 0.08	78.35 ± 1.88	243.44 ± 4.14	53.76 ± 1.99
B+1st+2nd L+I	8.60 ± 0.35	9.40 ± 0.30	79.21 ± 1.33	244.57 ± 2.32	54.06 ± 0.27
B+1st+2nd+3rd L+I	11.50 ± 0.37	10.00 ± 0.43	68.75 ± 0.41	220.22 ± 2.10	46.79 ± 1.73
BHT	20.80 ± 0.60	5.60 ± 0.09	-	-	-
EDTA	-	-	97.64 ± 0.58	-	-

[a]Values are mean ± SD (*n = 3*). [b]The half maximal effect concentration. [c]The effective concentration at which the absorbance is 0.5. B, Bud. I, Internodes. L, Leaves. GAE, Gallic acid equivalents. RE, Rutin equivalents. DE, dry extract. BHT, butylated hydroxytoluene. EDTA, ethylenediaminetetraacetic disodium salt.

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Eriodictyol and kaempferol: common plant flavonoids that exhibit anti-proliferative, anti-inflammatory and neuroprotective activity

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Aromatic and medicinal plants play an important role on human diet, due to the biological activities attributed to their chemical constituents. Phytochemicals exert several bioactivities, being the most evident the antioxidant activity, although other properties, such as anti-proliferative, anti-inflammatory, anti-diabetic, antiaging, are commonly attributed. In this study we aimed to evaluate the bioactive properties of two flavonoids, kaempferol and eriodyctiol, that are present in some medicinal and aromatic plants, such as, *Thymus pulegioides* [1], namely the anti-proliferative activity against RAW 264.7 cells (murine macrophages cell line), the anti-inflammatory activity using lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, and the capability to inhibit the acetylcholinesterase activity. The anti-proliferative activity was estimated with the cell viability assay using Alamar Blue indicator, as described [1]. The anti-inflammatory activity was assessed by the capacity of selected flavonoids to inhibit the production and release of nitric oxide (NO) by LPS-stimulated macrophages. The released NO was quantified using the Griess reagent, as described [2], and the activity of AChE was quantified as in [1] to assess the neuroprotective activity

Results show that: i) both flavonoids exhibited anti-proliferative activity against RAW 264.7 cells, being eriodyctiol more effective (IC50 ~ 60 µM) than kaempferol (IC50 > 200 µM); ii) at non-citotoxic concentrations, eriodyctiol was also more effective in inhibiting the NO released by RAW 264.7 cells (60% and 40% reduction, compared to control) than kaempferol; and that both showed a concentration dependent inhibition of AChE. These results favour the attribution of function foods designation to plants rich in these two flavonoids.

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Antimicrobial activity and phenolic profile of chestnut (*Castanea sativa* Mill.) by-products

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Different agro-industrial by-products have been described as potential sources of phenolic compounds, which have been reported to exhibit several properties, such as, antimicrobial, antioxidant, anti-inflammatory, antimutagentic and cardioprotective [1]. During chestnut (*Castanea sativa* Mill.) processing, a large amount of wastes are generated that could be used as interesting and cheap sources of these compounds [2]. Therefore, this study aimed to extract phenolic compounds from chestnut by-products of Longal variety and evaluate their antimicrobial activity against antibiotic resistant bacteria.

The phenolic compounds were extracted from different parts of the chestnut, namely the shell, inner shell and bur, as well as from the leaves of chestnut tree. The extraction was performed with 100% ethanol, which was eliminated by rotoevaporation at low temperature (40ºC) and the obtained residue was re-dissolved in DMSO to a final concentration of 100, 75, 50, 25 and 10 mg/mL for the antimicrobial activity assay. The antimicrobial susceptibility assay was performed using the Kirby-Bauer disc diffusion method against 6 Gram-positive bacteria: *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Listeria monocytogenes*; and 4 Gram-negative bacteria: *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. For the phenolic compounds analysis, the extracts were dissolved in ethanol and the analysis was performed by HPLC-DAD-ESI/MS.

The individual major phenolic compounds identified and quantified in the chestnut by-products were gallic acid, ellagic acid, trigalloyl-HHDP-glucose and quercetin, syringetin and myricetin glycoside derivatives. All the extracts showed antibacterial activity, with the inner shell's extract being effective against all Gram-positive and two Gram-negative bacteria. This extract presented syringetin-3-*O*-galactoside and myricetin glycoside derivatives as the main phenolic compounds. None of the extracts had antibacterial activity against *E. coli* and *S. enteritidis*, while *S. aureus* and *S. epidermidis* showed susceptibility to all tested extracts.

The obtained results show that chestnut by-products are a good source of phenolic compounds with antimicrobial activity, being a potential tool to potentiate antibiotic effects in combating multidrug-resistant bacteria.

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Recovery of bioactive compounds from Fucus vesiculosus algae by membrane technology and evaluation of biological action against cardiovascular diseases

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In this work the Fucus vesiculosus was study. This brown macroalgae is part of the diet in Asian countries and it is gaining acceptance in Europe and America, it can be consumed either raw or cooked, or supplemented in food products. These seaweeds have an interesting nutritional potential being rich in bioactive compounds, and among them the most important compounds found are phlorotannin's that show a high antioxidant capacity in vitro, especially those with 4 to 8 pholoroglucinol units. [1],[2]
The seaweed F. vesiculosos was collected in Tajus river (Lat. 38.7822 N; Long. -9,0913 W), and its study aimed the knowledge extension from this edible seaweed namely the targeting of hypercholesterolemia, which is a condition often associated to cardiovascular diseases, and it is one of the leading causes of death worldwide. In order to simulate the ingestion of the seaweed, different extractions were made such as gastric digestion, pancreatic digestion and complete digestion and, to simulate cooking a high pressure decoction. The extracts were analysed in terms of phenols, proteins, lipids, carbohydrates and mannitol. The biological activities such as antioxidant capacities, acetylcholinesterase inhibition and HMG-CoA reductase (the main enzyme from cholesterol biosynthesis pathway) inhibition were assayed and the best results were obtained from extracts after high pressure decoction.
Two ultrafiltration membranes, with different molecular weight cut-offs, 51445 and 10784 kDa, were prepared using the phase inversion method. Membrane technology was applied in diafiltration mode for further purification of the bioactive compounds present in the high pressure decoction. Two membrane experiments were carried out, the first using the 51445 kDa membrane, generating a concentrate stream (larger compounds) and a permeate. This last permeate was then permeated through the 10784 kDa membrane, generating a permeate (smaller compounds) and a concentrate (medium compounds). Permeates and concentrates containing different compounds were collected and, the results show that medium and small size compounds collected together have better biological activities.

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Stability of water soluble β-carotene-loaded nanoparticles

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Carotenoids is a group of pigments naturally present in vegetable raw material. They have biological properties and have been used mainly in food, pharmaceutical, and cosmetic industries. β-Carotene is the most common carotenoid in food, which plays an important role in metabolism and health care. However, the application of β-carotene is currently limited because of their poor water-solubility, high melting point, low bioavailability, and chemical instability [1-3]. The research into water-soluble carotene-encapsulated systems is important for their potential formulations and the production of carotene-enriched foods.

Water-soluble β-carotene / 2-hydroxypropyl-β-cyclodextrin complexes were prepared by co-precipitation method using a β-carotene in acetone solution and a cyclodextrin in water solution at the concentration of 25 %. The prepared water-soluble inclusion compounds were additionally complexated with pectins. For particles formation, pectins with the different degree of esterification (pectic acid, high methoxyl pectin and low methoxyl pectin) at different pH (4, 5, 6, 7) were used. The pectin concentration in the solution of resulting particles was constant and equal to 0.4 mg/mL while the complex concentration was equal to 0.02, 0.03 or 0.05 g/mL. FTIR, Raman spectroscopy, UV and DLS methods were used to confirm the interaction of components.

The studies of stability were based on the measurement of the changes of particle size during the storage and the estimation of the relative stability of β-carotene. To evaluate the stability of encapsulated β-carotene against UV radiation or temperature, the particles were irradiated by UV-C 30 Watt lamp or incubated at 60 and 95 °C temperature for certain period of time. Finally, the changes of absorbance at 450 nm were registered. It was evaluated how the stability depends on the ratio of components, the type of pectin and pH of the solution. It was observed that β-carotene in the particles with high methoxyl pectin had improved thermal and UV stability at the lower pH values. Meanwhile, based on size measurements, the particles with pectic acid were more stable at the higher pH values. The ratio of the components did not have a significant impact on stability studies, although at the lower concentration of inclusion complex in the particles, β-carotene had a slightly better UV stability.

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Characterization and stability investigation of Lycopene – Chitooligosaccharides complexes

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Lycopene is a potential bioactive natural compound found in fruits and vegetables, i.e. tomatoes, apricot, red grapefruit, cherry, pink guava, papaya, peaches and watermelon, and is responsible for their reddish-brown color [1]. It has unique structural and chemical features that may contribute to specific biological properties due to its conjugated double bonds [2]. Unfortunately, it is not stable against light, oxygen, high temperatures, acids and is water-insoluble [3]. The development of methods for increasing their solubility and stability is an important goal.

In this study, we report a successful mechanochemical method of lycopene (LYC) and chitooligosaccharides (CHIOS, Mn=3500 Da) complexation. CHIOS-LYC complexes were formed by kneading, sonification and lyophilisation techniques. The interaction of components and physicochemical characteristics of obtained particles, i.e. morphology, structure, size were investigated by ^{1}H-NMR, FTIR, XRD, DSC, SEM and DLS methods. Stability against light, temperature and pH of novel complexes were evaluated by UV and HPLC methods.

The phase solubility test revealed that CHIOS-LYC complexes are stable and stability constant K_S is 489 M^{-1}. They are soluble in water (3 - 11 mg/ml) and the yield of CHIOS-LYC complexes is in the range from 4 % to 48 %. The determined encapsulation efficiency varied from 10 % to 60 %. The hydrodynamic size of the complex particles in the aqueous solution was between 84-400 nm. Polidispersity index (PDI) was from 0.2 to 0.9. The analysis of ^{1}H-NMR, FTIR, XRD and DSC provided the evidence of LYC interaction with CHIOS at the molecular level and the complex formation. The SEM experiments revealed visual changes in the surface morphology of CHIOS-LYC complexes. The investigation of relative stability under various conditions indicated that about 30 % of LYC degraded at 4°C and 25°C in the dark. At the day light, 40% of LYC degraded at 25°C temperature within 30 days of storage at different pH values. The size of complexes and PDI under all storage conditions for 30 days changed insignificantly. Therefore, the complexation of LYC with CHIOS strongly increases its stability towards light irradiation, temperature and pH conditions. Novel CHIOS-LYC particles could be important LYC delivery systems for the food industry and the formulation of new type supplements.

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Screening of phenolic compounds in açaí and evaluation of its anti-angiogenic activity

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The açaí fruit (*Euterpe oleracea* Mart.) is a natural source of natural antioxidants, mainly attributable to its high content in phenolic compounds, with potential applications as functional food and nutraceutical. Phenolic compounds are well-known by their antioxidant, anti-inflammatory, anti-proliferative and cardioprotective effects [1]. Also, phenolic compounds are able to modulate angiogenesis, the process through which new blood vessels grow from pre-existing ones and a hallmark in diseases as cancer, diabetes and cardiovascular diseases [2].

This study aimed at the screening of phenolic compounds by HPLC-DAD-ESI-MS^n from a hydroalcoholic extract prepared from freeze-dried powdered açaí berries, and the evaluation of its angiogenic effects. The bioactivity of the açaí extract was evaluated using HMEC-1 cells (extract concentration ranging from 1 to 75 mg/L) for 24 h and assessed by cell viability (MTS), proliferation (BrdU incorporation) and formation of capillary-like structures (matrigel assay).

The profiling of the main phenolics from the hydroalcoholic açaí extract by HPLC-DAD-ESI-MS^n allowed the identification of various classes of phenolic compounds, namely the anthocyanin cyanidin-3-*O*-rutinoside, the flavanonol aromadendrin, as well as the flavone derivatives apigenin and luteolin glycosides. The bioactivity studies demonstrated that the hydroalcoholic extract of açaí exhibits anti-angiogenic properties by reducing proliferation and capillary-like structures at concentration of 10 mg/L, without cytotoxic effect. This work provides new insights to the development of a useful hydroalcoholic extract with nutraceutical properties and potential health benefits, namely against angiogenesis-associated pathologies.

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Development of bioactive emulsions from Cape-hake and pea protein: antioxidant and anti-hypertensive activities

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The production of fish protein hydrolysates is one of the alternative technologies for the upgrading of fish by-products. Numerous studies have shown that fish protein hydrolysates possess many bioactive properties making them a desirable ingredient in some foodstuffs. Thus, they have been used as ingredients in various types of food products with different objectives. Taking advantage of the known antioxidant and anti-hypertensive activities of fish protein hydrolysates, they were used in the current work in pea protein emulsions. Therefore, the objective of this work was to evaluate the antioxidant and anti-hypertensive activities of Cape-hake protein hydrolysates (HPH) in emulsions prepared with mixtures of pea protein isolate (PE) and HPH. These emulsions were prepared with a total protein content of 4% (w/w), 65% (w/w) of sunflower oil and 31% of water and the protein fraction had the following proportions: 100PE/0HPH, 75PE/25HPH, 50PE/50HPH and 25PE/75HPH. The color parameters, the antioxidant (DPPH and ABTS scavenging activity, reducing power and iron and copper chelating activity) and anti-hypertensive activity (inhibitory activity of Angiotensin-Converting-Enzyme, ACE) of the different emulsions were measured.

The emulsions prepared with the different proportions of HPH and PE showed a rheological behavior and texture characteristics similar to other food emulsions. Regarding the color of emulsions, the addition of increasing levels of HPH resulted in lighter emulsions and decrease of chroma and hue values. The protein fraction of all emulsions exhibited antioxidant activity. The highest antioxidant activity was generally recorded in the protein fraction of emulsion 25PE/75HPH (Table 1).

Table 1. DPPH and ABTS radical scavenging activity and Fe^{2+} and Cu^{2+} chelating activity of emulsions prepared with different proportions of PE and HPH.

Emulsion	DPPH (% inhibition)	ABTS (% inhibition)	Reducing power (Abs 0.5)	Fe^{2+} chelating activity (% inhibition)	Cu^{2+} chelating activity (% inhibition)
100PE/0HPH	21.3±1.68^{a}	15.4±0.62^{a}	0.100±0.02^{a}	77.8±2.65^{a}	31.8±0.61^{a}
75PE/25HPH	38.8±0.22^{b}	63.9±0.93^{b}	0.240±0.02^{b}	92.4±0.20^{b}	53.1±0.41^{b}
50PE/50HPH	42.5±0.88^{c}	76.2±1.49^{c}	0.310±0.00^{c}	91.5±1.85^{b}	73.3±0.15^{c}
25PE/75HPH	44.9±2.50^{c}	85.2±1.28^{d}	0.370±0.01^{d}	89.3±3.64^{b}	72.3±0.15^{c}

Different letters in each column denote significant differences between emulsions.

The ACE inhibitory activity was in the range 15.4-77.1%, respectively for the 100PE/0HPH and 25PE/75HPH protein fractions.

These results may conclude that HPH can be used in this type of products allowing to valorize fish by-products and to produce healthy innovative food.

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Bioaccessibility of bioactive components from vegetal liquid foods

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Vegetal foods play a fundamental role in the diet since, in addition to nutrients, contain bioactive compounds that provide beneficial physiological effects, by modulating specific body functions [1]. As a result of some studies that link these compounds directly with health [2], the food industry develops new foods that incorporate bioactive compounds in their formulation. However, in order to exert their biological activity, the bioactive compounds should pass through the intestinal barrier, that is, be bioaccessible for the organism [3]. In this sense, it is important to know how much of the bioactive compound that is ingested is finally absorbed by our organism. This topic has aroused the interest of the scientific community and, although there is already a lot of information available, it is often contradictory.

The aim of this work was to contribute to the study of the bioaccessibility of some bioactive compounds present in foods of vegetable origin with different composition in macro and micronutrients. An *in vitro* digestion system has been adapted to measure the bioaccessibility of bioactive compounds, specifically in liquid food. The *in vitro* digestion protocol was developed with orange juice (*Citrus sinensis* var.Navelina) and with a microalga (*Chlorella vulgaris*) suspension. The evolution of the total phenolic compounds in the gastric and intestinal stages of the digestion was studied and the intestinal bioaccessibility was calculated. Due to the liquid state of the foods, the oral phase was not considered. Nutrient's absorption in the intestinal phase was simulated through a dialysis membrane.

The results showed that both, the stability of the phenolic compounds throughout the *in vitro* digestion, and their bioaccessibility, depend on their chemical nature [2] and on the food matrix. In this sense, for both samples the phenolic compounds decreased significantly by 20% during the gastric digestion stage. However, after the intestinal step, a significant total loss of 47% of phenols in the orange juice and a 34% increase in the microalgae suspension was observed, with regard to the content before the digestion. In the dialyzed fraction, which is the one that can be absorbed, a total phenol content of 13,3 ± 0,2 mg gallic acid / 100 mL orange juice and 479 ± 78 mg gallic acid / 100 g microalga was recovered, which meant a bioaccessibility of 21% and 40%, respectively. The greater bioavailability of the phenols in the microalga could be related, in addition to its different nature, to the different interaction with the rest of the macronutrients present in it [4, 5].

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Impact of chalcones on reactive species production by human neutrophils in hyperglycaemia conditions

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Diabetes *mellitus* (DM), a chronic metabolic disorder of multiple etiology, is one of the world's largest epidemics of the 21th century. Several epidemiological studies reported that inflammation has an important role in the development and aggravation of DM. Neutrophils, the body's first-line-defence cells, are involved in the maintenance of the inflammatory response under hyperglycemic conditions, due to their sustained production of high levels of reactive species (RS) [1]. The excessive RS production by human neutrophils in DM contribute to a harmful imbalance between the RS production and their removal by antioxidants, a condition that is commonly designated as oxidative stress (OS). Consequently, OS actively contributes to the development and worsening of DM, being responsible for the development of several diabetic complications [2]. Therefore, there is a growing scientific interest to find alternatives to the currently used anti-diabetic drugs, able to modulate OS and improve the DM condition. Chalcones are plant-derived compounds that present a 1,3-diaryl-2-propen-1-one scaffold. Chemically, they are open chain flavonoids with a three-carbon α,β-unsaturated carbonyl system joining the two aromatic rings (A and B). Due to their wide distribution in nature, simple chemistry, diversity of substituents, easy synthesis and innumerable biological activities, these compounds are scientifically sound and have been extensively studied [3].

The aim of this study was to evaluate the ability of a panel of 25 structurally related chalcones [varying the number and position of several substituents (hydroxyl, methoxyl, methyl or chlorine)], to modulate RS production by human neutrophils, in physiological and in hyperglycemic conditions, simulating DM status. For this purpose, human neutrophils were isolated and the production of RS was stimulated with phorbol 12-myristate-13-acetate, in media with two glucose concentrations, 5.5 and 30 mM. RS were detected by a chemiluminescent probe, luminol [4].

The chalcones exhibited an interesting modulatory effect against RS production, for some compounds the IC_{50} values being < 5 μM, and the majority presented similar effects in physiological and diabetic conditions. It was also concluded that the chalcones structure, more precisely the position and number of hydroxyl and methoxyl substituents, are determinant factors for the intended effect. The most active chalcone presents a hydroxyl group at position 2' at the A ring and a methoxyl and a hydroxyl groups at positions 3 and 4, respectively, at the B ring. These preliminary results suggest that chalcones may be promising compounds to be used as an adjunctive treatment of DM.

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Ellagitannins, antocyanins and polysaccharides in fruits of Purple Queen® pomegranate cultivar grown in substrates with dredged sediments.

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Pomegranate (*Punica granatum* L.) is classified among the top seven fruits with the highest beneficial properties for humans. Most of the pomegranate health benefits are related not only to the intake of the juice, but also to its ellagitannins [1] distributed in the exocarp and mesocarp, commonly named peel. Pomegranate peel is the richest source of ellagitannins, and comprises 40-50% of the whole fresh fruit. Pomegranate peel contains a great amount of bioactive polysaccharides (mainly pectin) that have shown antioxidant effects[2], immunomodulatory [2] and prebiotic properties[3] *in vitro*, immunomodulatory and anti-inflammatory effects [X]. Among the cultivated varieties, Purple Queen® is a new one particularly appreciated for its precocity ripening time, approx. in second half of August. This peculiarity is notably functional for the marketing strategy since it broadens the availability of pomegranate fruits during the year. Melgarejo et al. observed that fruits cultivated in growing media with remediated sediment showed an increase of aryls juice soluble compounds[4], but not reported findings on anthocyanins and ellagitannins profiles, neither on the polysaccharide content in fruit.

Objective of this work was to verify the effects on the nutraceutical components of fruits from plants growing in pot on different % of remediated sediments. The study was carried out determining anthocyanins in juices and ellagitannins and polysaccharides in peel. To this aim, fruits were collected in 2017 and 2018 from plants grown only in remediated sediment or in a mixture 1:1 with peat-based substrates. The juices were obtained by a commercial extractor suitable to gently work at low temperatures, while the decoction was chosen as suitable procedure to recover ellagitannins and polysaccharides from peel. The phenolic concentrations were determined by HPLC-DAD and expressed with respect to the juices or on dry peel. The yield in polysaccharides were gravimetrically evaluate after dialysis of the whole fractions and freeze drying. These sample were than analysed by Size Exclusion Chromatography to determine the apparent molecular weight of the main constituents.

The anthocyanins profiles in juice resulted partially modified: cyanidin glycosides proportionally increased with the increment of the sediment percentage as well as the total anthocyanin content. The total ellagitannins in peel showed an increase up to 37% in plants cultivated only in remediated sediment when compared to those grown only in peat. Analogously polysaccharides increased proportionally to the % of the added sediment with amount doubled in the fruits grown on 100 % of remediated sediment. The analyses by SEC for all the samples collected in 2018 showed very similar profile, suggesting the structure of the main constituents remained unmodified.

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Assessing anti-oxidant, anti-proliferative and anti-inflammatory properties of red seaweeds (*Grateloupia turuturu* and *Porphyra umbilicalis*) coupled with a phenolic characterization

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The red seaweeds *Grateloupia turuturu* and *Porphyra umbilicalis* are often used for human consumption, with emphasis in East Asia. However, studies focusing their bioactivities and correlation to nutraceutical action are lacking. Aiming to fulfil this gap, hydroethanolic and aqueous (infusion and decoction) extracts of *G. turuturu* and *P. umbilicalis* were prepared and used to assess their: i) anti-proliferative activity against RAW 264.7 cells (a murine macrophage cell line); ii) anti-inflammatory activity (at non-cytotoxic concentrations; using lipopolysaccharide stimulated RAW 264.7 cells); iii) their content in total phenols, *ortho*-diphenols and flavonoids; and iv) anti-oxidant activity (assessed against $ABTS^{\bullet+}$, $^{\bullet}OH$ and $NO^{\bullet}$ radicals). The cell viability and anti-proliferative activity was assessed with the alamarBlue® assay [1]; the anti-inflammatory activity was assessed by measuring the nitric oxide (NO) released using the Griess method, as described [1], the total phenols, *ortho*-diphenols, flavonoids and anti-oxidant activity were assessed by colorimetric methods [e.g., 1, 2]. Results show that total phenols and *ortho*-diphenols contents were higher in the aqueous extracts (for both species). Residual flavonoids contents were observed for all extracts. The aqueous extracts demonstrated the greatest $ABTS^{\bullet+}$ scavenging activities; the antioxidant properties against $^{\bullet}OH$ were more accentuated for the hydroethanolic extracts; *G. turuturu* aqueous extracts developed the highest protection against $NO^{\bullet}$. Anti-proliferative activity was dependent on exposure time and extracts concentrations, with *P. umbilicalis* aqueous extractions exhibiting the greatest activity. The *G. turuturu* aqueous extracts showed better anti-inflammatory properties than the corresponding hydroethanolic extract; however, the highest anti-inflammatory activity was shown by the *P. umbilicalis* hydroethanolic extract. These results reinforce the potential of these seaweeds as functional food.

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Protective activity of olive phenolic compounds sulfate metabolites against red blood cells oxidative injury

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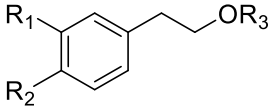
Bioavailability studies have shown that the main polyphenols found in olive oil, namely hydroxytyrosol and its esteres, are efficiently absorbed in the small intestine. Therefore, the potential health benefits of hydroxytyrosol and its derivatives maybe attributed to both parental compounds and their phase I and phase II metabolites. The growing interest in the bioactivity of natural polyphenols and of their metabolites requires pure metabolites to be used in bioassays and as standards in research protocols. Therefore, we report here the synthesis of some hydroxytyrosol and tyrosol metabolite sulfates.[1].

Red blood cells (RBC) are particularly exposed to oxidative damage. However, they have poor repair and biosynthetic mechanisms, suffering oxidative lesions whenever oxidative stress develops. Whenever the hemoglobin is released from RBCs it is potentially dangerous and play a major role in pathophysiology of cerebral haemorrhage, blast pressure injury and myocardial ischemia reperfusion injury.

This study aimed to investigate the protective effects of sulfate metabolites against the RBC oxidative injury [2]. Some of the metabolites tested, namely hydroxytyrosol 1-O-sulfate, the mixture of hydroxytyrosol 3'-O and 4'-O monosulfates and the mixture of hydroxytyrosol acetate 3'-O and 4'-O-monosulfates, were found to protect erythrocytes from AAPH-induced oxidative haemolysis after 2 and 4 h of incubation in a dose dependent manner. However, the introduction of a sulfate group into the molecule at the 3'-O or 4'-O positions of the ring decreased the protective activity of hydroxytyrosol in a significant way. The haemolysis studies were in agreement with the results observed in the analysis of cellular density performed by optical microscope.

Table 1 - Synthetized Compounds.

Compound		R_1	R_2	R_3
	1	OH	OH	H
	2	OH	OH	$COCH_3$
	3	H	OSO_3H	$COCH_3$
	4	OH	OH	OSO_3H
	5	OSO_3Na	OH	$COCH_3$
	6	OH	OSO_3Na	$COCH_3$
	7	OSO_3Na	OH	H
	8	OH	OSO_3Na	H

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Innovative functional foods from grapes and *Vitis* waste

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This work represents a model of circular viticulture that aims to development by-products of wine industry, with attention to green technologies for the recovery of biocomponents from grape pomace for implementation of a "closed cycle" system to reuse the waste derived from wine processing, to extract ingredients and biological fractions for design new bio-products from *Vitis vinifera* L., standardized in bio-components for food, nutraceutical industry and phytotherapy. Aim of this work is to design of new food products made by grapes, concentrated musts and standardized extracts of *Vitis vinifera* like new ingredients and biological fractions to obtain new sustainable products. [1] Many studies on the phytochemical composition of grapes are reported and the main constituents of *Vitis vinifera* L. belong to the class of polyphenols, consisting of anthocyanosides, catechins, flavonols and stilbenes. Anthocyanins are responsible for the red colour of many fruits and flowers and play a fundamental role in the plant in preventing damage caused by UV radiation. The anthocyanosides of grapes are mainly represented by the 3-O-monoglucosides [2,3]. Sagrantino is one of the grape variety with the highest quantity of polyphenols, anthocyanins and natural sugars. The production area covers 670 hectares in Umbria region in the centre of Italy. This cultivar is mainly used for the production of wine which is protected by the DOCG mark (of controlled and guaranteed origin) since 1992. Grape pomace consists mainly of peels (skins), seeds and stems and accounts for about 20 –25% of the weight of the grape crushed for wine production [4]. In this work, the content of total polyphenols and total anthocyanins of grape pomace form Sagrantino wine production, of juices of Sagrantino grapes and of grape jams was evaluated by HPLC-DAD analysis and also the total antioxidant capacity was determined with Folin-Ciocalteau spectrophotometric method. Seeds and skins are valuable raw materials for extraction of polyphenols, in particular grape skin extracts are also being marketed due to their more complex structure consisting of anthocyanins from skins and procyanidins from seeds. The aqueous phenol extracts are clarified and concentrated as thick liquids or spray dried to powders. These products are already in the trade as natural colours and approved by the FDA for colouring of beverages [5]. In addition to phenolic antioxidants, grape pomace also contains significant amount of lipid, proteins, no digestible fibre and minerals.[4] Experimental studies indicate that grape polyphenols could reduce atherosclerosis by a number of mechanisms, including inhibition of oxidation of LDL and other favourable effects on cellular redox state, improvement in endothelial function, lowering blood pressure, inhibition of platelet aggregation, reducing inflammation and activating novel proteins that prevent cell senescence. [6] Grape pomace powder analysis form Sagrantino cultivar showed anthocyanins content of 1.2-1.3 mg/g and total phenols 12.9-18.3 mg/g. The cold pasteurized grape juice had a content in anthocyanosides of 918-946 mg/L (mainly cyanidin 3-*O*-glucoside) and the total antioxidant capacity of about 373 mg GAE/100ml. The total anthocyanosides of grape jams are 294-430 mg/L (cyanidin 3-*O*-glucoside). The results obtained in this work allowed to hypothesize a possible use of Sagrantino pomace powder as an innovative ingredient to add to grape juices and grape jams as a source of functional compounds.

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Dairy products as alternatives to reduce glycemic index of wheat bread

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Chronic metabolic diseases, such as type 2 diabetes mellitus, are recognized as an important public health problem and there is a considerable interest in reducing the glycemic index (GI) of foods. Bread is a staple food widely appreciated and consumed in large quantities worldwide, with an important role in human nutrition. However, elicits a high glycemic response, thereby increasing the risk of metabolic diseases. Dairy products (DP) are widely used in the baking industry due to their beneficial effect on the technology aspects and functional properties. In addition DP are reported as low GI (GI≤55), releasing glucose at a slower rate compared to high GI foods (GI≥70), that could be an interesting alternative to decrease starch hydrolysis rate on baked goods and reduction of GI.
In this work, the impact of the yoghurt (Yg) and curd cheese (Cc) addition (from 6% up to 22% w/w) on starch rheology behaviour (SRB), in vitro starch digestibility, and in the prediction of GI of the breads was studied. Mixing and heating-cooling cycles (MicrodoughLab equipment, Sweden) were performed to evaluate changes on starch rheology behaviour. The rate of starch digestion and estimation of glycemic index (eGI) were determined based in vitro starch digestibility by enzymatic methods [1]; [2]. Higher incorporations of Yg (Fig1.A) and Cc (Fig1.B) promoted different impacts on SRB, compared to control dough (CD): for Yg, the results suggested that the caseins may have a incompatibility interaction with starch molecules, which upon cooling become entrapped in the gelled starch matrix, by exopolysaccharides-amylose-casein interactions, probably due to the depletion flocculation mechanism [3]; In the case of Cc, a thermodynamic incompatibility between denatured albumins and starch, hindering a matrix structuration, was observed. These results were attributed to the dilution effect of starch granules and a certain competition for water by the Cc milk proteins. These different effects were clearly reflected on the GI of the breads (Fig1.C), with a significant reduction of 34% for Yg and 42% for Cc, resulting in breads with intermediate-low (55-69) GI, compared with control bread (CB).

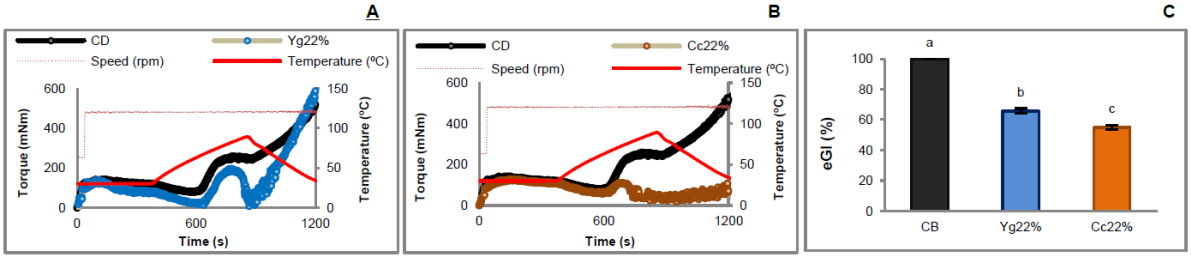

Fig. 1. Effect of Yg (A) and Cc (B) addition on starch rheology behaviour and eGI of the breads (C).

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Halophyte plants as potential tool against toxicity of food contaminants

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Foods can be contaminated with toxic chemicals such as residues resulting from agricultural activities, components from food packaging or compounds formed during food processing and cooking at high temperatures [1]. The long-term consumption of contaminated foods can cause adverse effects such as increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), promoting oxidative stress and inflammation in the gastrointestinal tract [2]. These events can cause oxidative damage to cellular constituents and potentially contribute to the development of diseases such as cancer [3].

The oxidative state caused by exposure to toxic chemicals present in food may be attenuated by the consumption of products rich in antioxidant phytochemicals such as (poly)phenols. Accordingly, plant-based foods rich in (poly)phenols, like fruits and vegetables, have been linked to lower cancer risk, mostly related to those of the gastrointestinal tract [4]. Hence, there has been an increasing effort to find efficient antioxidants that could prevent oxidative stress-related diseases, without causing deleterious effects. Plants exposed to constant abiotic stresses could be promising sources of antioxidant molecules, which is the case of halophyte species that live in saline conditions but can simultaneously be exposed to drought and intense ultraviolet light. In addition, many halophytes are consumed in the Mediterranean diet, which suggests that halophyte-based products could be safely consumed.

In this work, twelve extracts of halophyte species from the south of Portugal were evaluated for their antioxidant properties by testing their capability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide radicals. The species that exhibited higher antioxidant capacity were tested *in vitro* for their chemical composition by determining total phenol, ortho-diphenol and flavonoid contents. The best antioxidant performance was displayed by *Armeria pungens* (Link) Hoffmanns. & Link*, Pistacia lentiscus* L., *Carpobrotus edulis* L. and *Polygonum maritimum* L.*,* with the two latter species showing the best free radical scavenging capacity and also the higher content in total phenol and ortho-diphenol compounds. These results suggest that *P. maritimum* and *C. edulis* are good candidates to include in the human diet, either as (poly)phenol-rich food or as part of functional foods, to attenuate oxidative stress and inflammation generated along the digestive tract potentially involved in the development of malignancy.

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Enzymatic synthesis of lactulose using a novel biocatalyst

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Functional food is defined as 'natural or processed foods that contain known biologically-active compounds which provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age [1]. Many compounds of animal and plants origin can be used to complement functional foods, but nowadays, the most commonly used are probiotics, prebiotics and antioxidants [2]. Prebiotics are 'substrates that are utilized by host microorganisms conferring a health benefit' [3]. They have been successfully incorporated in a wide variety of food products like breads, baked goods, nutritional bars, meet products, salad dressings, sweeteners and yoghurts [2]. One of the most well-recognized prebiotics is lactulose, a disaccharide derived from lactose. Lactulose is not found naturally so it has to be synthetized through different methods, e.g. chemical or enzymatic synthesis, and electro-activation [4]. Traditional enzymatic synthesis involves the use of β-galactosidases or glycosidases. However, a problem associated with the use these enzymes is an eventual lactulose degradation by the biocatalyst and the simultaneous production of monosaccharides and galacto-oligosaccharides, which compromises the yield and purity of the final product [4]. Recently, the production of lactulose through lactose isomerization catalyzed by cellobiose 2-epimerase was reported [5]. This new strategy is gaining more attention as a preferable methodology for industrial usage due to the noteworthy yields obtained. In this study, we propose a new and promising biocatalyst for lactulose production. The combination of a GRAS producer such as *Saccharomyces cerevisiae* with a production process using only lactose as substrate can be a more economic and attractive approach for the synthesis of lactulose for functional food applications. The biocatalyst was obtained by cloning the cellobiose 2-epimerase gene from *Caldicellulosiruptor saccharolyticus* in the BY4741 *S. cerevisiae* strain. The cellobiose 2-epimerase enzyme was produced in a synthetic media composed by YNB (0,67 g/L), glucose (10 g/L) and amino acids (100 mg/L). After 24h of fermentation, the biomass was disrupted, and the supernatant was used for lactulose production. Following an optimization of several reaction parameters (reaction buffer, pH, time and substrate concentration), the best condition led to a prebiotic yield of 20.8% and a productivity of 8,83 g/Lh^{-1} after 1h of reaction. Based on this result, it is possible to conclude that this approach could be a promising and safe strategy for lactulose production, since the biocatalyst was obtained in only 24h and it was able to reach a good prebiotic yield and productivity, being competitive with the reported ones involving the cellobiose 2-epimerase enzyme produced by *E. coli* that reaches 57% of prebiotic yield after 4h reaction [6]. Additionally, the GRAS status of *S. cerevisiae* confers another great advantage to the process. Furthermore, when comparing the results with the β-galactosidase enzyme, higher yields and productivity were obtained in shorter fermentation and reaction time. A study using β-galactosidase reports the production of lactulose in an enzymatic membrane reactor reaching a maximum yield of 5,47% [7]. Afterwards, the potential of this biocatalyst for the synthesis of lactulose was studied and it was demonstrated that it could be a sustainable and safe approach to produce lactulose suitable for food application.

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New insights in the microencapsulation of polyphenols for functional foods – encapsulation, characterization and *in vitro* release studies of oleuropein loaded microparticles

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The worldwide recognition of the potential health benefits of olive oil consumption had promoted its production and consumption [1]. It is recognized that olive oil acts in the prevention of cardiovascular diseases and as a cancer therapy coadjutant. Furthermore, olive oil consumption may benefit the lowering of blood pressure and also may reduce DNA and lipid oxidation. It is also described its positive effects on inflammation processes and as well as in endothelial dysfunction [2]. The positive health benefits of olive oil consumption are related to the presence of a specific polyphenol – oleuropein. This bioactive compound can differentiate normal cells from cancer cells, inhibiting the proliferation and promoting the apoptosis only in cancer cells [3]. The positive effects of oleuropein for breast and prostate cancers have been intensively studied using cellular models [4].
However, the amount of oleuropein is considerably low in extra virgin olive oil and refined olive oil due to its hydrolysis into tyrosol and hydroxytyrosol during olive oil processing [6].
Therefore, considering the health benefits of oleuropein and its low resistance to hydrolysis during olive oil production, its incorporation in polymeric shells may protect this bioactive compound from hydrolysis when it is incorporated in food matrices as a polyphenol source.
Moreover, when formulated in microparticulated systems, oleuropein can be controlled released in the food matrix, excreting, therefore, a powerful antioxidant action. Additionally, this bioactive compound can be released from microparticles during the gastrointestinal tract, which may confer the health benefits associated with its consumption.
Oleuropein-loaded microparticles were obtained by water-in-oil-in-water double emulsion solvent evaporation technique as described by Paulo and Santos [5]. Microparticles were characterized regarding their morphological characteristics, particle size distribution, encapsulation efficiency, loading content, product yield, thermal and oxidative stability. Moreover, in vitro release studies were performed in a simulated gastrointestinal tract. It was verified its adequate thermal and oxidative protection, which indicates that when encapsulated and incorporated in food matrices, oleuropein can be adequately protected from the food environment.

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Assessing the antigenotoxic potential of wild-caught and aquacultured macroalgae (*Ulva rigida*) alongside a phytochemical profiling – a dietary assay with *Drosophila melanogaster*

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In the last years, marine macroalgae have been defended as functional food, due to their bioactive compounds, conferring them several beneficial properties (*e.g.* antibacterial, immunomodulatory, anti-inflammatory and anti-tumour) [*e.g.* 1]. Nevertheless, macroalgae genoprotective potential was scarcely investigated, albeit some signs about their antioxidant, antigenotoxic and antimutagenic capacities [*e.g.* 2]. *Ulva rigida* is a green edible alga, globally distributed and easily grown in aquaculture, with a rich nutritional composition, though variable according to the growing conditions, which, in turn, may influence its properties. Some studies reported the antigenotoxicity of *U. rigida* extracts [3] and the antioxidant potential of *U. lactuca* extracts [4], highlighting that *Ulva* species may strengthen genome protection. Hence, the current study intended to investigate the antigenotoxic potential of two *U. rigida* batches - wild-caught and aquacultured - alongside their phytochemical profiling.

For that purpose, the antigenotoxic potential was assessed in *Drosophila melanogaster*, through the adoption of the comet assay, following a dietary exposure to wild-caught and aquacultured *U. rigida*. Two supplementation levels (2.5 and 5%) of each algae origin were tested, in the presence and absence of streptonigrin (SN; a mutagenic agent). The phytochemical profiling (semi-quantitative determination) was achieved by gas chromatography - mass spectrometry.

In general, aquacultured *U. rigida* displayed higher levels of the compounds identified, especially fatty alcohols, sterols, sesquiterpenoids and glycerol esters. Regarding the antigenotoxic potential against basal damage (in the absence of SN), slight variations were depicted, since 2.5% wild-caught alga induced a decrease on DNA breaks, while the same supplementation level of aquacultured specimens showed the opposite, highlighting an origin-based difference. On the other hand, when *D. melanogaster* was challenged by SN, both *U. rigida* origins showed to efficiently decrease the genotoxic damage and, particularly, 5% aquacultured alga depicted a higher antigenotoxic potential, reinforcing an origin-based pattern. The algae growth conditions proved to induce differences on the phytochemical profile, which cannot be dissociated from the different antigenotoxic potencies observed. It can be suggested that higher amounts of sterols and sesquiterpenoids are related to the stronger antigenotoxic action of aquacultured algae against SN. Overall, a diet supplementation with *U. rigida* promoted genome protection in *D. melanogaster*, particularly against SN-induced damage. These findings contribute to the reinforcement of the concept of algae as functional food.

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POSTERS

Food Packaging

Mechanical, thermal and structural characterization of ethylene vinyl alcohol copolymer films containing betalain-rich beetroot

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In recent years, demand for minimally-processed, preservative-free foods with longer shelf-lives has increased significantly. As a result, the food industry is focusing on the modification of food packaging as one possible solution to meet this need. The development of new packaging films that use bioactive substances obtained from natural resources that enhance food quality is currently growing (Tawakkal et al., 2014). However, the addition of bioactive compounds to the packaging must not compromise the integrity of the film. Therefore, optimal structural, mechanical and thermal properties must be maintained for food packaging applications. The present study was therefore aimed to develop an antioxidant film based on EVOH combined with betalain-rich beetroot using a casting procedure (Muriel-Galet et al., 2012). Two different preparations of red beet (powder and extract) were investigated with levels of 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5% (w/w), and were incorporated into EVOH films with attention focused on the thermal (DSC and TGA), structural (FTIR), and mechanical properties.

The red beet was found to adequately disperse in the copolymer in both the powder and extract forms, without detriment to the thermal stability or mechanical properties. The resulting films were also slightly more crystalline, had a higher resistance to UV light and significant antioxidant properties (Fig. 1). The water barrier properties were slightly reduced, particularly in the case of the powder form of the additive, which could potentially be minimized by using these films in multilayer formulations or coatings. Although the powdered form of the red beet contained some insoluble components, there were no significant benefits obtained by incorporating the extract form of the red beet when compared to the powdered form. The results show some promise for food packaging applications where antioxidant activity is required with the added benefit of barrier and UV properties.

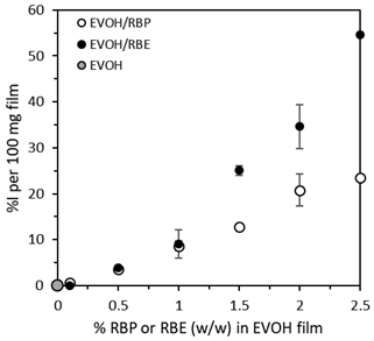

Fig.1. Percentage of antioxidant inhibition of EVOH control film and those containing different proportions of RBP and RBE.

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The suitability of potato starch and peels wax for biobased thermoplastics production

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During potato processing, several biomolecule-rich by-products are generated and wasted (1,2), opening an opportunity for their valorisation. In this work, potato industry by-products were used as raw materials for bioplastic production (3). Starch and waxes were recovered from potato washing slurries and potato peels, respectively. The influence of waxes concentration (0.5-2.5% w/w of dry starch weight) in the melt tenacity and extrudability of thermoplastic starch-based formulations was studied. Each thermoplastic material was obtained through melt-mixing. The addition of wax revealed to decrease the torque during melt-mixing, although the removal of glycerol appears to rise it in the final phase of mixing, when the temperature reaches 120 ºC and consequently the water disappears from the mixture. The addition of 0.5% (m/m_{starch}) of wax increased twofold the mass flow rate in relation to the pristine starch bioplastic, meaning that extrudability was enhanced by the presence of small quantities of wax. The humidity content of the filaments was higher in the bioformulations with added waxes (up to 1.7%; 0.8% higher than the control), indicating that wax acts as a moisture barrier. These biobased materials were injected to obtain tensile test specimens to study mechanical and wettability properties. The presence of wax in the starch matrix produces materials with higher flexibility, and greater hydrophobicity than the other materials. In this way, potato peels, a by-product from the potato industry, is valorised by enhancing thermoplastic starch properties, originating sustainable bioplastics for several applications, such as food packaging.

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Potato chips byproducts to produce thermoplastic biogranulates

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Potato chips industry generates wastes with biomolecules of interest for the development of thermoplastic biobased materials, namely starch-rich slurries and oil residues. Starch has been used for bioplastics production due to its thermoplastic properties, film forming ability, and biodegradability. Nevertheless, starch materials have poor mechanical resistance and humidity tolerance, which makes necessary the incorporation of other biomolecules to develop improved starch-based materials. The use of oil recovered from frying residues may allow to overcome those drawbacks. In this work, starch was recovered from industrial potato washing slurries by freeze-drying and sieving. In addition, oil was extracted from potato frying residues through Soxhlet extraction with *n*-hexane. Afterwards, starch and oil were combined to produce thermoplastic biogranulates using a melt-mixing technique. The influence of oil on melt-flow index, tenacity, humidity content, and heat capacity of starch-based granulates was studied as well as in the mechanical performance and wettability of the corresponding injected test specimens. The addition of oil increased the fluidity and the humidity content of starch-based granulates. Moreover, starch/oil specimens revealed to have more flexibility, elasticity, and hydrophobicity than the granulates without oil. Therefore, potato chips byproducts are a source of biomolecules of interest to produce thermoplastic biogranulates with improved mechanical performance and wettability.

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Using potato and egg industry byproducts on the development of bio-based films

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Agrofood industries produce many wastes worldwide [1]. The large amount of waste produced represents a great loss of valuable materials and, on the other hand, severe environmental and economic management problems [2]. However, many of these residues have compounds with potential to be reused as raw materials for biobased films. Aiming to prompt this hypothesis, with benefit for both sectors, in this work, starch and calcium carbonate were recovered from potato chips and egg processing industry byproducts, respectively, and were physicochemically characterized for further use for bio-based films production.

The recovered starch shows an amorphous structure with a granule size of around 66 μm, and its granules have a heterogeneous shape, varying from spherical to oval, like commercial potato starch (73 μm and with analogous starch granules). In addition, the recovered starch evidences a gelatinization temperature of 60 ºC and an enthalpy of 12.5 J/g, similar to commercial starch (gelatinization temperature of 66 ºC and an enthalpy of 11.2 J/g). The recovered starch shows the same ATR-FTIR bands than the commercial ones.

The recovered calcium carbonate presents a crystalline structure and has a granule size of around 130 μm, higher than commercial calcium carbonate (9 μm). ATR-FTIR analysis shows that recovered calcium carbonate recovered from the eggshells has a spectrum profile similar to the commercial one.

When applied on films production, recovered starch allows to produce stretchable and somewhat hydrophilic films, but when in the presence of calcium carbonate, the mechanical performances and hydrophobicity of starch-based films are improved.

Therefore, potato and egg processing industry byproducts reveal to be interesting sources of compounds for the development of bio-based films, opening an opportunity for their valorization while developing new biodegradable plastics for packaging sector.

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Enhancing shelf life of fresh figs (*Ficus carica* L.) using edible coatings based on chitosan and alginate emulsions with olive oil

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Fresh figs are very appreciated and have been associated with health benefits [1]. However, these fruits are highly perishable [2]. In this study, edible coatings were studied envisaging their positive effect in enhancing figs shelf-life.

Fig fruits were harvest at 'green ripe' stage and coated with emulsion based edible coatings produced with 2% w/v chitosan and 1% w/v sodium alginate and olive oil (25% and 100% biopolymer basis, respectively). After coatings application by immersion and drying, the coated fruits were sprayed with crosslinking solutions (tripolyphosphate (TPP) 6% w/v and calcium chloride 1% w/v, for chitosan and alginate based films, respectively). The coated fruits as well the control, were maintained at 4 ºC and analyzed after 1, 7, 14 and 19 days of storage. After each time interval, fruits were maintained at 25 °C for 2 days in order to simulate consumer handling conditions. The physiology (CO2 production) and quality attributes (fungal decay, color, weight loss, firmness, total soluble solids (TSS) and titratable acidity (TA)), were evaluated.

The main results have shown that coatings were effective on delaying postharvest ripening indicators [3], such as softening, mass loss by evaporation and respiration rate (Fig.1). In addition, the edible coatings delayed TSS production and TSS/TA ratios (fruit maturity index), which increased significantly in control fruits associated with the over-ripening and senescence processes (Fig.1). Results from physiology and quality parameters suggested that the maximum shelf life period for control fruits was 7 days at 4 °C plus 2 days at 25 °C, while the coated figs could be stored with optimal quality, and delaying ripening up to 19 days at 2 °C plus 2 days at 25 °C.

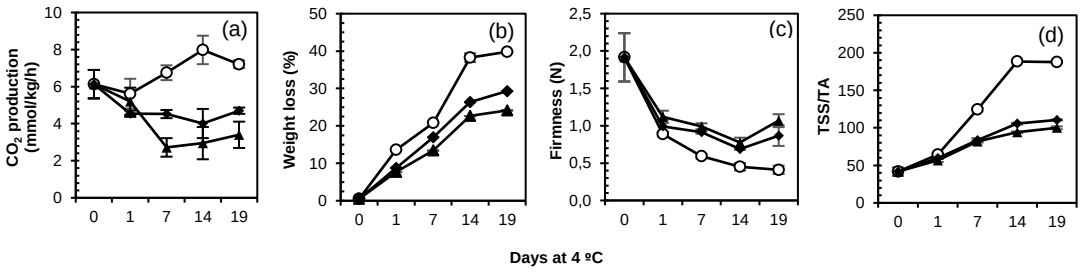

Fig.1. Evolution of figs fruit physiology (a) and quality (b,c and d), coated (◆ Ch/O-TPP; ▲ Alg/O-Ca2) and uncoated (O control), after cold storage and analyzed after 2 days at 25 °C. Data are the mean ± SE.

The applied edible coatings have demonstrated to be very effective in enhancing shelf life of fresh figs. Future work will be focused on the combination of coatings application with a designed compostable package.

Acknowledgments: This work was partially funded by the PhD scholarship granted to Tiago M. Vieira by University of Lisbon (2015). Support from LEAF (Linking Landscape Environment Agriculture and Food) research center also acknowledged.

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POSTERS

Food Processing

Influence of high-voltage electric discharge on acrylamide and HMF content in cocoa husk

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Cocoa husk is a by-product of chocolate industry. Usually, it is separated from cocoa bean after the roasting and prior to other operations of cocoa bean processing. It represents app. 10 – 15 % of the bean and presents great problem regarding disposal. In order to explore it's potential for application in food, it's safety must be determined. Acrylamide is arising as a potentially harmful food component, since it is linked with higher risk of developing cancer in all age groups. It is found in food processed above 120 °C at low moisture, where it forms as a product of Maillard reactions.

During roasting, cocoa husk is exposed to temperatures as high as 135 °C for as long as 55 min, it contains starch and proteins and is potentially significant source of acrylamide and HMF. Therefore, the aim of this research was to determine the content of acrylamide and HMF in cocoa husk separated after the roasting cocoa bean at 135 °C for 55 min and to investigate possibility of reducing their content in the husk by high-voltage electrical discharge (HVED). Acrylamide was determined by UPLC-MS/MS and HMF by HPLC-DAD.

The results showed that HVED treatment may be applied to reduce the content of acrylamide and HMF in cocoa husk below the limit of quantification, even below the limit of detection in some cases.

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Production of mixed beverage of milk and açaí fermented by kefir with added soluble fibres

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Kefir is a fermented beverage obtained by fermenting milk or sugary solutions using milk kefir grains (MK) or water kefir grains (WK). Such grains are symbiotic cultures of microorganisms, consisting mainly of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeast (Y). The beverages obtained are slightly carbonated, rich in organic acids and vitamins, besides being low alcohol content and present probiotic and prebiotic characteristics (depending on the culture and the substrates utilized). Other substrates such as fruits and vegetal extracts can also be successfully applied to the production of kefir fermented beverages, increasing additional value and sensory modifications. The addition of soluble fibres, such as fructooligosaccharides (FOS) or inulin (I), may add prebiotic character to these beverages. The aim of this study was to evaluate the production of mixed beverages of milk and *açaí* (typical Amazonian fruit) fermented by MK and WK with the addition of soluble fibres (FOS and/or I). Four formulations containing 50:50% m/m of milk:*açaí* were evaluated: (1) without addition of soluble fibres, (2) with 4 %w/w of FOS, (3) with 4 %w/w of I and (4) 2 %w/w of FOS and 2 %w/w of I. A condition utilizing only cow milk was added to the study for comparison purposes. All formulations were made in triplicate for both kefir cultures. The fermentations were conducted in 100 cm^3 beakers, with substrate volume of 50 cm^3, without stirring, at 25 °C for 24 hours. The cellular growth (X g / 100 g), beverage yield (Y, g/100 g), CO_2 production (g/100 g), concentration of soluble solids (SS g/100 g), pH, acidity (total and volatile) (TA and VA, g/100 g) and concentration of protein (P, g/100 g) were measured. The results obtained varied considerably in function of the conditions utilized, with (g/100g) X = 8.79 to 39.31, Y = 90 (average), CO_2 = 6.06 to 10.70, SS_f = 4.0 to 12.5, TA = 0.19 to 0.63, VA = 0.03 to 0.24 and P = 1.91 to 3.03. The final pH ranged between 3.30 and 5.38. *Açaí* and inulin were the variables that affected the process the most. The increase in the concentration of *açaí* caused a reduction of X, SS, pH and P and increase of TA and VA, whereas the increase in the concentration of inulin increased X and SS and decreased TA. The increase in the concentration of FOS caused an increase of SS, VA and reduction of pH. Regarding the type of grain utilized, the main differences observed were that the use of MK increased the TA while WK caused an increase of X. Besides the characteristics measured, the addition of *açaí* alters the colour of the beverage, which acquires the purple colouration of the fruit. The results obtained in this study are in accordance with previous results in which the potential of the use of MK and WK cultures for the fermentation of a mixed beverages of milk and *açaí* was evaluated [1]. Furthermore, it was possible to verify the influence of the addition of FOS and inulin as soluble fibres in this type of beverage devising the production of a symbiotic drink. It is also important to highlight that this study contributes to the incorporation of *açaí* in processed foods, which is positive from both the economic point of view, as it adds value to this typical fruit from the Amazon and from the nutritional point of view, as the *açaí* nutrients are incorporated in different types of products.

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Influence of culinary practices on lipid and protein oxidation in cooked chicken burgers

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Chicken meat is a valuable source of nutrients, such as essential amino acids and polyunsaturated fatty acids (PUFAs), and several important micronutrients. Meat thermal treatment, mandatory before consumption, triggers the generation of reactive oxygen species (ROS) and consequent oxidation of food constituents. In particular, PUFAs oxidation results in the formation of lipid oxidation products (LOPs), such as malonaldehyde (MDA), hexanal (HEX), and 4-hydroxy-2-nonenal (HNE). MDA and HNE are involved in different pathologies such as metabolic and neurodegenerative diseases, and cancer [1]. Aminoacids are also oxidized during cooking, increasing protein denaturation and aggregation due to the formation of disulfide and dityrosine bridges, and leading to the formation of Schiff bases, resulting in the loss of amino acids. The addition of spices and herbs has been shown to be effective against the formation of LOPs during food thermal treatment, as well as red wine polyphenols have also shown protective effects against oxidation [2]. However, the influence of beer on protein and lipid oxidation remains unknown. Moreover, the formation of these oxidation compounds seems to increase during *in vitro* digestion, mostly in the acidic environment of the gastric phase which has been described as bioreactor of lipid peroxidation [1].

In this work we have studied the influence of adding oregano (0.2 % w/w) and beer (1mL/8 g meat) prior to microwave (700 W) and oven cooking (200 ºC) of chicken burgers on lipid and protein oxidation by measuring the formation of MDA, HNE, HEX, carbonyls, and Schiff base structures. MDA and carbonyls were measured spectrophotometrically at 532 and 370 nm, respectively. HNE and HEX were quantified by HPLC-FLD, and the emission fluorescent spectra of Schiff base structures was recorded between 390 and 600 nm. The *in vitro* digestion of samples was performed according to the internationally standardized method proposed by COST Infogest network [3].

Results showed that cooking significantly increased LOPs (MDA, HEX, and HNE) in comparison with the raw controls, whereas protein oxidation (accessed as carbonyls content and Schiff base structures) does not seem to be potentiated by cooking. Addition of oregano significantly decreased MDA, HEX, and HNE formation, in comparison with cooked control samples (oven and microwaving), while adding beer did not influence the formation of LOPs, but increased Schiff base formation. The *in vitro* digestion increased MDA; carbonyls, and Schiff base structures regardless of the culinary practices, while HNE and HEX contents were only increased in chicken burgers cooked with oregano. Despite this increase, cooking with herbs was the culinary practice with the lowest content of HNE, HEX, MDA, carbonyls, and Schiff base structures either after cooking or *in vitro* digestion, suggesting that adding oregano prior to cooking may be used as a mitigation strategy to reduce the oxidation of lipid and proteins products during cooking, as well as reducing the formation of these hazardous compounds throughout *in vitro* digestion.

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Preparation of processed-meat flavorings without animal fat

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Processed-meat flavorings are widely used in foods such as instant noodles, hams, sausages, and quick-frozen dumplings in China. Fat is the common material used in preparation of processed-meat flavorings, but it can lead to consumers' dislikes on the flavored food due to greasiness and high-calories from the fat of the flavorings. The research object was to prepare processed-meat flavorings with vegetable oil instead of animal fat. Palm oil and rapeseed oil (1:9 w/w) was heated at 130 °C for 2 h while stirring and blowing of air at 1.5 L/min into the oil. By sensory evaluation and electronic nose, the oxidized oil obtained in peroxide value (PV) of 150 meq/kg was found with the odor characteristics similar to a heated lard. The minced lean meat of pork in water (1:1 w/w) was hydrolyzed with first Protamex (0.4% w/w) at 55 °C for 3 h, then Flavourzyme（0.3% w/w）at 55 °C for 1 h. The enzymes were inactivated at 85 °C for 10 min, and then the supernatant of the enzymatic hydrolysate after centrifugation was lyophilized. Both the oxidized oil and the dried powder of enzymatic hydrolysate were used in the thermal reaction of 120℃ and pH6.5 for 1.5 h. The recipe for the thermal reaction was mainly 3 g of pork hydrolysate, 0.1 g of glucose, 0.05 g of xylose, 0.04 g cysteine, 0.03 g glycine, 0.03 g of V_{B1}, 0.1 g salt, 0.08g of MSG, 0.015g of five spice, and 0.03 g of oxidized oil. Sensory evaluation showed the reaction product had a stewed pork broth flavor but had no visible fat floating and greasiness. By GC-MS and GC-O, the key aroma compounds in the reaction product were revealed to be 2-methylthiophene, 2-methyl-3-furanthiol, 3-ethylpyrazine, 2-pentylthiophene, 3-thiophenecarbaldehyde, nonanal, octanal, hexanal, 2, 5-thiophenedicarboxaldehyde, furfural, anethole, etc. The research results help to overcome the defect of the current meat-flavoring products and upgrade their preparation technology.

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Effect of cooking on aroma profiles of Chinese foxtail millet (*Setaria italica*) and correlation with sensory quality

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The effects of roasting, boiling, and freeze-drying after boiling on volatile aroma compounds in three varieties of Chinese foxtail millet (Setaria italica), namely, Jingu 21, Fenghonggu and Dongfangliang were determined. During boiling significant ($p < 0.05$) increases in the contents of several unsaturated aldehydes, alcohols, and benzene derivatives were observed, unlike roasting that mainly increased in the contents of pyrazines. Freeze-drying after boiling decreased complexity of flavors with a reduction in the contents of volatile compounds. Descriptive sensory analysis showed that the maximum intensity of 'popcorn-like' and 'smoky' odors was observed for roasted samples, whereas boiled and pre-boiled-freeze-dried samples were characterized by 'boiled rice' and 'boiled potatoes' odors, respectively. A correlation of odor-active profile data with descriptive sensory analysis clearly established the role of pyrazines such as 2-ethyl-3,5-dimethylpyrazine in contributing to the 'popcorn-like', 'boiled beans', and 'smoky' odors, whereas dienals such as (E,E)-2,4-decadienal were responsible for the 'boiled rice' aroma.

Effects of Different Storage Conditions on kernels Quality and Processing Characteristics of Oats

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Abstract：Oats with rich nutrition and health function were aged easily during storage. In order to explore the changing of nutritional quality and oat dough characteristics of Oats in the process of aging,the content of water, ash, fat, protein, starch and fatty acid was measured by comparing the new oat kernels with oat kernels stored at room temperature and 4℃ for one year. The effects of storage temperature on the characteristics of oat dough after oat kernel aging were investigated under different water addition conditions.

The results showed that the content of conventional nutrients decreased after storage of oat kernels for one year. The nutrients of grains stored at room temperature decreased more and the content of starch, water and fat decreased significantly. After storage, the content of main fatty acids in oat kernels decreased. Oleic acid, linoleic acid, MUFA and PUFA decreased significantly after storage at room temperature($p<0.05$).With the increase of water content,the dough hardness decreased during the year, and the elasticity and cohesiveness increased. Under the same water addition conditions, the oat grains after storage had higher dough hardness than the new oat dough, and the elasticity and cohesiveness were lower than the new oat dough. After storage at room temperature, the dough of the sample has higher hardness and lower elasticity and cohesiveness.

Effect of enzymatic treatment on the content of anthocyanins and polyphenols in blackcurrant (*Ribes nigrum* L.) juice and press-residue

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Blackcurrant (*Ribes nigrum* L.) is an important fruit crop in Estonia, being cultivated on more than 380 hectares [1]. Blackcurrant fruits are known to be rich in health promoting bioactive compounds, mostly anthocyanins and other polyphenolic compounds. Depending on the cultivar, the technological properties and quality of fruit are quite different according to fruit skin thickness, fruit size, biochemical composition etc [2],[3], but juice quality depends on processing technologies as well [4]. Enzyme-aided processing is used to increase juice yield, and to affect the extraction of bioactive compounds into the juice [5].

The aim of this study was to determine the effect of pre-pressing enzymatic (pectinase) treatment of blackcurrant fruit mash on the juice yield and content of polyphenolics in juice and press-residue. The fruits of cultivars ‘Ben Lomond’, ‘Pamyati Vavilova’ and ‘Karri’ were collected at the experimental plots of Polli Horticultural Research Centre of Estonian University of Life Sciences (58 °7'26"N, 25°32'43"E) and stored at -20°C until processing. Frozen fruits were thawed at room temperature for 24 h, crushed mechanically, and juice was extracted using water press (max pressure 3 bars).

The enzyme treatment of mash improved juice yield up to 44% depending on the cultivar. The total content of polyphenols (incl. anthocyanins) in juice was not significantly different in treated and non-treated samples. Still, the contents varied according to the cultivar and treatment showing up to 29% increase of polyphenols in enzymatic press-residue, and up to 18% decrease of anthocyanins in enzymatic juice in comparison with freshly frozen berries.

The results of the pilot scale experiment revealed that enzyme-aided juice pressing will give higher juice outcome as expected, which does not necessarily mean increased contents of bioactive compounds in juice. Better exploitation and maximum valorization of press-residue rich in polyphenolic compounds, especially anthocyanins is an important issue for reducing agricultural wastes and the loss of health promoting bioactive compounds.

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The influence of keeving on the profile of volatile compounds and terpenes in fermented apple beverages

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Keeving is a process that involves removing from the apple juice the nutrients, among others amino acids, by binding them with pectins. As a result of the method, fermentation occurs slower and stops spontaneously. This process is not commonly used in the production of alcoholic beverages.

The aim of the study was to evaluate the impact of keeving process on the concentration of volatile compounds in the fermented apple beverages.

The musts used for the experiments were made of the Rubin apple cultivar. Pectinesterase (Univar, Poland) was added to the ground apples and left for 24 hours to separate the juice and pectin. The must was pressed (Zottel hydropress), which was then poured into fermentation containers and $CaCl_2$ was added in three concentrations (0.1, 0.2 and 0.4 g/l). Must was inoculated with various yeast strains: wine (Elegance), cider (Gozdawa), distillery (RED Ethanol) or wild (Wild & Pure). The process of fermentation was carried out for 30 days at 20°C, during which time a brown cap (chapeau brun) was collected and fermentation continued. A fermented must not to be subjected to keeving was a control. In the obtained fermented beverages the profile of volatile compounds, e.g. alcohols, esters and terpenes, was determined using a gas chromatograph with a FID detector.

Among the terpenes, isoeugenol, pinocarveol and terpen-4-ol dominated. The synthesis of terpenes depended on the yeast strain used. Distillery yeast synthesized more pinocarveol, terpen-4-ol and β-damascenone than other strains, but less eugenol and isoeugenol. The smallest amounts of terpene compounds were found after the fermentation of settings by wild yeast. A significant influence of keeving on the level of the majority of terpenes was also demonstrated. In all attempts after defecation an increase in concentration of camphor, eugenol and β-ionone was observed. The concentration of terpen-4-ol after the use of keeving decreased in all attempts. Pinocarveol concentration also decreased in all samples except those fermented with distillery yeast, in which the amount grew. No effect of $CaCl_2$ dose on the content of terpenes in beverages was observed.

The group of other volatile compounds was dominated by amyl alcohols, ethyl acetate (especially in samples fermented by wild yeast) and isobutanol. Keeving caused a reduction in the content of amyl alcohols (in samples after fermentation with wild yeast), isobutyl acetate and ethyl hexanoate (in some attempts until complete disappearance). However, after the defecation, an increase in the concentration of some of the analyzed esters was observed. Particularly large differences (2-16-fold) were observed in the case of ethyl octoate and phenylethyl acetate. Smaller differences were noted for ethyl acetate and diethyl acetal. The dose of $CaCl_2$ added during defecation did not have a significant effect on the amount of volatile compounds in beverages. However, a significant influence of the amounts of $CaCl_2$ on the concentration of phenylethanol and isobutyl acetate has been demonstrated (especially in fermented samples involving cider yeast).

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Change mechanism in the gel properties of frozen whole egg liquid during frozen storage

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The frozen whole egg liquid has some outstanding advantages such as longer shelf life, more convenient transportation, and simple production process, which can retain the nutrients of fresh eggs to the greatest extent as well as regulate the balance between supply and demand of the off-season and peak seasons for shell egg sales. Frozen storage can cause changes in the gel properties of frozen whole egg liquid, which affects its application in food[1].

In the present study, the gel properties and physicochemical properties of frozen whole egg liquid during frozen storage at -18 °C up to 180 d were dynamic monitored. The results show that the gel hardness and water holding capacity of frozen whole egg liquid reduced by 36.12% and 18.28% respectively, and the gel micro-network structure changed to loose and porous. In particular, the most obvious the decrease of gel properties was the most significant during the 20-120 d of frozen storage. The pH of frozen whole egg liquid increased from 7.68 to 7.80 in the first 30 days of the frozen storage, and then significantly decreased to 7.20 when frozen storage for 180 days. The protein solubility decreases gradually during freezing storage, especially in 10-90 days. Moreover, total sulphur and surface sulfhydryl content reduced by 32.01% and 42.40% respectively, while surface hydrophobicity increased. The average particle size of frozen whole egg liquid continues to grow as the frozen time increases, combined with the electrophoresis results, indicating that some protein aggregation. Meanwhile, the DSC results showed that the thermal stability was reduced.

These results suggested that frozen storage induced in whole egg protein structure change and protein intermolecular aggregation. This is the main reason why the gel properties of whole egg are reduced. Meanwhile, these results were consistent with some reporters about other animal proteins[2,3]. The study explored the change mechanism in gel properties of frozen whole egg liquid during the frozen storage and provided the theoretical basis for further improvement of its gel properties.

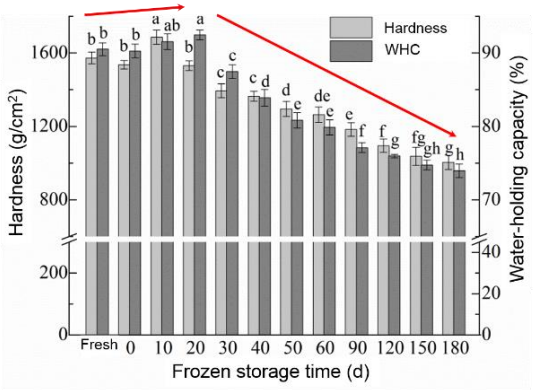

Fig.1. Gel hardness and water holding capacity of frozen whole egg liquid during freeing time

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Study on shelf-life and quality characteristics of highland barley fresh noodles as affected by microwave treatment and food preservatives

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Highland-barley wet fresh noodle (HBFN) was obtained by mixing highland barley flour and wheat flour, and had a good freshness and unique flavor. But it is an easily perishable food for high initial microbe quantity, moisture content and abundant nutrient substances, therefore prolong its shelf-life is the major concern of food industry. Accordingly, the purpose of this study was to reduce the initial microbe quantity in the mixed flour and inhibit the growth of microorganisms in HBFN by adding ε-PL (0.04%) combined with different concentrations of calcium propionate (CP) while it was stored at 25℃.

The mixed flour was treated in a microwave oven at 800W for 0 s, 10 s, 20 s, 30 s, 40 s, 50 s and 60 s, and noodles were made with ε-PL and different concentrations of CP, and then stored in sterile plastic bag at 25℃. Then moisture content was measured by gravimetric method, and microbe was determined according to the standard GB4789.2—2016. Textural properties were measured by texture analyzer, and the transverse relaxation time T_2 was determined using a CPMG pulse sequence by LF-NMR.

The results showed that microwave treatment (800W) for 40 s provided 30% reduction of initial total plate count (TPC) of the mixed flour without decline of noodle quality. The shelf-life of HBFN treated with MW+ε-PL (0.04%) + CP (0.020% / 0.025%) reached 80 h and 88 h respectively, and it keeps a good hardness, elasticity and chewiness compared with the control group. The ratio of peak area of the weakly bound water and free water increased, while it decreased for the strongly bound water. In a word, the results point out that the “hurdle effect” is a practical and economical approach, promising technique to prolong the shelf-life of highland barley fresh noodles.

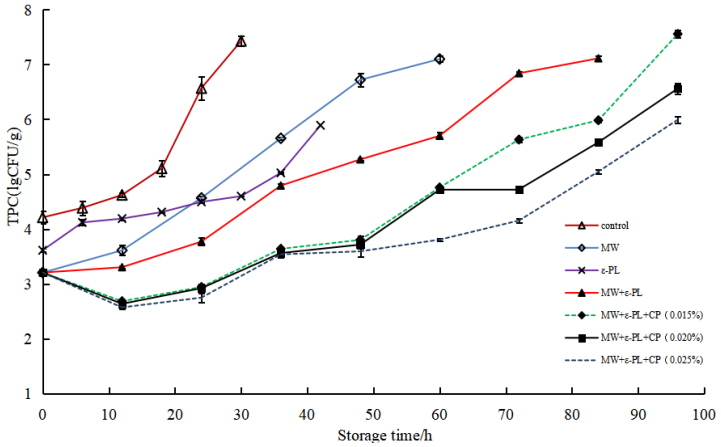

Fig.1 TPC changes in HBFN with different treatment during storage at 25℃

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Quality Characteristics of Sweet Potato Powder and Rice Cake according to Various Processing Methods of Sweet Potato Cultivar

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Sweet potato powders were produced from different cultivars, and their quality characteristics were analyzed and processing aptitude was evaluated by adding it to Korean traditional 'rice cake'. Sweet potato used in this study were developed by the Bioenergy Crop Research Institute, National Institute of Crop Science, Rural Development Administration, Korea and using sweet potatoes were mealy type 'Sinyulmi', sticky type 'Pungwonmi', colored 'Sinjami'(purple) and 'Juwhangmi' (orange). Methods for making powder were as follows: at first sweet potatoes were washed & cut into pieces and applied the four methods- freeze drying, hot air drying, hot air drying after roasting (referred as HR), steaming after hot air drying (referred as SH) and then pulverized to prepare powder. The color, moisture content, sugar content, total polyphenol content, free sugar and DPPH activity of the powder were analyzed and the rice cake were analyzed for pH, hardness and total polyphenol content. The L value of 'Sinyulmi' showed 86.2 and the fresh sweet potato was 84.2. The freeze drying showed similar color to that of fresh. The a value of 'Sinjami' was 19.9 for fresh sweet potato, 19.0 for SH. The color was similar to that of fresh sweet potato. The b value of 'Juwhangmi' was the highest at 40.8 in fresh, and freeze drying, hot air drying, HR and SH showed 21.5, 28.0, 28.3 and 30.2, respectively. Moisture content of mealy type was higher than that of colored and sticky type in freeze drying, but there was no significant difference among hot air drying, HR and SH. Regardless of the cultivars, the highest degree of sugar content was found in SH. The total polyphenol content was determined by freeze-drying which showed 'Sinyulmi' 169 mg, 'Pungwonmi' 290 mg, 'Juwhangmi' 332.7 mg and 'Sinjami' 1,288 mg per 100g. The highest value of 'Sinjami' was 1,404.9 mg by hot air drying, 1,086.6 mg of HR, and 1,867.7 mg per 100g of SH. The total polyphenol contents were not significantly different in the freeze drying, hot air drying, HR of 'Sinyulmi', 'Pungwonmi' and 'Juwhangmi'. Free sugar content was the highest at 31.1 g per 100g in the hot air drying of 'Pungwonmi' and the lowest showed by freeze drying. The activity of DPPH was similar to that of freeze drying and SH.

On the other hand, when sweet potato powder added to rice cake, the pH of rice cake showed similar value, regardless of the processing method. The hardness of rice cake was high as 185.1 g and 80.2 mg per 100g of total polyphenol content of SH. The hardness of 'Pungwonmi' was hot air drying 172.4 g and HR 169.1g, and the total polyphenol contents were 90.36 mg and 97.1 mg per 100g, respectively.

Acknowledgments: This work was conducted with the support of The Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01133201), Rural Development Administration, Republic of Korea

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The impact of the roasting process on chemical composition and sensory profile in specialty coffee from Colombia

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Introduction: During the roasting process cascade of chemical reactions accrue, from which non-volatile compounds degrade and form new compounds with potential aroma, flavour attributes. Meanwhile, the roasting process has a negative influence on the health-beneficial phenolic compound composition in coffee. There is limited research done on how the roasting process impacts both – chemical composition and sensory profile of specialty coffee. It is important to understand the balance point of the roasting process when the sensory profile and health-beneficial phenolic compounds are at the best ratio in specialty coffee. The aim of the research was to evaluate the chemical composition and sensory profile changes during the roasting process in specialty coffee.

Material & Methods: Coffee from Caldas–Chinchina in Colombia was roasted in Latvia specialty coffee roastery in three different roasting levels. The moisture, pH, acrylamide, amino acid and fatty acid profile, total phenolic and flavonoid content and the volatile compound profile was analysed in roasted coffee samples. Sensory evaluation was performed by Specialty Coffee Association (SCA) certified cupping team using the SCA cupping protocols.

Results: The total phenolic and flavonoid content decreased with roasting level. Also, the individual phenolic compound composition was strongly influenced by the roasting level, showing the highest chlorogenic acid, caffeic acid and epicatehin concentration in light roasted coffee. The sensory description of coffee samples matches with volatile compound odour characteristics. Coffee roasted at light roast level was valued with the best specialty coffee characteristics – pleasant acidity, fruity and floral notes like pineapple, dried apricot, elderflower. From the volatile compound profile point of view furaneol (pineapple-like odour) and benzyl alcohol (floral, rose odour) was detected only in light roast coffee. The unpleasant volatile compound (2-methoxy-4-vinylphenol, 2-methoxy-phenol and nonanoic acid) presence in dark roasted coffee influenced the sensory profile. The dark roasted coffee was characterised as bitter-harsh, smoky and phenolic.

Conclusion: The sensory quality and phenolic compound concentration decreased with increasing roast level. The light roasted coffee showed pleasant aroma and flavour characteristics both from the sensory and volatile profile point of view. The medium roasted coffee presented more caramel and roast-like characteristics and the dark roasted coffee showed smoky phenolic notes. Both in medium and dark roast level phenolic and flavonoid concentration in coffee was lower than in light roast. The light roast level was stated as the most suitable for specialty coffee roasting.

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Influence of processing and the incorporation of algae on the nutritional profile of a whole wheat pasta

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Pasta products are worldwide consumed and well-accepted by all age groups due to their sensory and nutritive value, low cost and easy preparation. Traditionally manufactured with durum wheat semolina and water, the new trends in food industry, as a result of consumers demand, include pasta enrichment with healthy ingredients to enhance its nutritional (high-protein sources from non-animal origin) and/or functional properties (antioxidant, anti-hypertensive or anti-inflammatory effect) [1]. Recently, algae and seaweed-based products have received increasing attention due to their nutritional contribution for human diet, being also one of the most promising sources of bioactive compounds. For instance, the microalgae *Spirulina* has been reported as a high-quality protein source, rich in essential amino acids, minerals, vitamins (vitamin B12 and pro-vitamin A), antioxidants and a biomass with potential application as a natural food pigment [2]. The brow seaweed *Himanthalia elongata* is an interesting source of nutritional and bioactive compounds such as soluble fibre, iodine, proteins, polyphenols and carotenoids [3].

Nowadays, several foodstuffs that include algae as ingredients are available on the market (eg. pasta, cookies, bread, desserts, beverages, etc…). Knowing that food enrichment and processing can lead to changes on the nutritional value of food, the aim of this study was to evaluate the proximal composition of a whole wheat pasta with or without the incorporation of *Himanthalia elongata* (2%) and *Spirulina* (1.5%), before and after the conventional pasta processing (boiling in water for 15 minutes).

The results showed an increase in the protein content of the algae-based pasta (13% dw) when compared with the same pasta without algae (10% dw), probably as a result of algae incorporation. Indeed, *Spirulina* showed a high protein content (59% dw) which represents a theoretical contribution of 0.89% for the total protein content of the pasta with algae. Total dietary fibre (47% dw), which 30% is soluble and 17% insoluble one, is the major nutritional component of *H. elongata*. However, no significant differences (p>0.05) were found in the fibre content when the pasta was enriched with 2% of *H. elongata*. Pasta processing of two different pasta lead to a decrease of all the nutritional parameters analysed: ~ 50% of protein, ~ 42% of ash and ~ 48% of total fibre (fw).

Acknowledgments: To ALGAS ATLÂNTICAS ALGAMAR (Pontevedra, Spain) for providing the samples. The work was supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national funds. S.M.F. Bessada and R.C. Alves thank to FCT the PhD grant SFRH/BD/122754/2016 and the CEECIND/01120/2017 contract, respectively.

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How pre-treatment freezing influences some quality parameters of the sous-vide cooked chicken breast

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Using fresh, high quality ingredients is essential in the food industry. Sous-vide technology is a popular advanced heat treatment method, it gives exceptional organoleptic properties to the products. This cooking method amplifies both the high quality parameters and also the substandard raw material. Carefully chosen, good ingredients will be even better and substandard ingredients will be worse.

In our study we examined the color (CIELab: L*, a*, b*), the texture (P2/n needle on an SMS Texture Analyser XT plus device; hardness value was determined by measuring the maximal force at the 20 mm puncture test), the weight loss (∆m%), and pH (not shown) as quality parameters of the chicken breast meat. The effect of pre treatment storage and the effect of the sous-vide cooking was evaluated. Chicken breast samples were kept refrigerated (3 days and 10 days at 3 °C after slaughtering) and frozen (10 days and 6 month at -25 °C) and were cooked sous vide (45 minutes at 65 °C) after each storage period. Meat samples were cooled to 10 °C in iced water after the heat treatment. The measurements were carried out the same day. ANOVA and CDA was used to process the data.

Weight loss was highly determined by the pre treatment storage period. It grew from 5,8 % (3 days at 3 °C) to 14,9 % (6 month frozen). Freezing increased weight loss and longer storage time (at both temperatures) had a slight effect as well. Lightness (L*) showed no difference between the samples. Yellowness values (b*) increased while redness (a*) decreased as longer storage period and freezing was applied before heat treatment. A slight increase (though not significant) in hardness was observed at the raw 6 month frozen sample compared to the refrigerated samples however the same 6 month frozen sample was less tough after the sous-vide cooking. TBA values of the raw meat samples slightly increased during the storage period at both temperatures, in contrast to the sous-vide cooked samples where the TBA numbers showed significantly higher values at the meat samples kept for longer periods at 3°C and at -20°C.
Evaluating all measured data CDA analysis points on the important difference between frozen and refrigerated samples. Storage time as awaited has more important role at the +3 °C stored samples. The 10 days storage (until the 'use by' date) seems to be too long, as the measured quality parameters of the chicken breast showed important changes and the sous-vide treatment amplified these differences. In the 1st and 2nd function determined CDA space these samples were clearly separated from the other groups. Our work proved that pre treatment storage method and storage time do have effect on the final product - freshness is decisive.

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Macroalgae-fortified Frankfurters and *"Chouriço"*: chemical analysis and comparison of preservation processes

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In addition to the growing formulation of functional foods, the food industry pursues the application new preservation methodologies that, besides safety, also guarantee improved sensory and nutritional characteristics of the products in comparison to the traditional processes [1]. In this context, high-pressure technology appears as an emerging technique, with distinct food products already launched in the market [2]. Because of their high and balanced nutritional values, macroalgae are being introduced as ingredients in new food products at exponential rates [3].

The present work intends to evaluate possible chemical changes (on moisture, protein, total fat, ash and minerals) introduced by using a macroalgae mixture as an ingredient in a meat and vegetarian frankfurters and in a traditional meat Portuguese product "*chouriço*". Moreover, the impact of non-thermal high-pressure processing on the quality and shelf-life of the new formulated food products was evaluated through surface colour and microbiological monitoring, by a period of storage of 45 and 180 days, in sausages and "*chouriço"*, respectively.

Notably, the use of macroalgae as ingredients mainly impacted the levels of minerals in the new formulated products, which had an overall increment in Mg, K, Ca, Mn and Fe contents and a significant decrease in Na/K ratio. As regard to high-pressure processing, our results showed that this was suitable to maintain the quality of the products. In fact, the application of this preservative method allowed to extend the shelf-life of frankfurters by more than twice and to improve the safety of "*chouriço*" by keeping its microbial load below the detection limit, while that of non-pressurized samples was about 2 Log_{10} CFU/g in. Regarding colour, the L*, a*, b* parameters remained constant over time in all samples, except for "*chouriço*" at 180 days, in which the reddish tonality decreased in comparison to that of the non-pressurized sample.

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Development and characterization of natural Hydroxytyrosol-rich extracts

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Olive oil production leads to 15% of oil and 85% of by-products. From the huge amount of olive waste produced, the need to add value to this natural residue arose [1].

Hydroxytyrosol (HT), is an important natural bioactive polyphenol found mainly in the wasted olive pomace. Its consumption has been linked to the benefits of the Mediterranean diet, and in particular to the protection against cholesterol oxidation and cardiovascular disease.

The present work uses a patented environmentally friendly technology based on purely physical process to obtain hydroxytyrosol-rich extracts [2]. The extraction process is based on a physical separation by membrane filtration - nanofiltration and a concentrate step by a reverse osmosis. The nanofiltration was tested using polymeric membranes with two different cut-offs (200-400Da and 150-300Da), getting an increase of the productivity using nanofiltration membrane with high cut off. The process was followed by a concentration step using reverse osmosis.

The concentrated natural extracts were characterized in terms of their stability along time, being observed that the HT concentration remains stable along 9 months. Moreover, the physical-chemical characterization was evaluated by the determination of the presence of total carbohydrates by the colorimetric “phenol-sulfuric” method, from a modified procedure and published by Masuko *et al*. (2004) [3]. This colorimetric method allowed to get sugar concentrations expressed in g/L in equivalent glucose. It was observed the presence in the extracts of carbohydrates with a range of concentration between 0.37-5.72g/L.

The integrated process allows to obtain olive extracts 100% natural hydroxytyrosol-rich for distinct high valuable formulations, namely for the production of nutraceutical and functional food.

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Influence of processing on the nutritional profile of Kombu seaweed

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Algae have been part of the human diet for thousands of years, especially by several oriental cultures (Japan, Korea and China). However, beyond their traditional consumption, a global demand for seaweeds is emerging due to their nutritional value and potential health benefits [1]. According to the FAO reports, algae farming is practised in about 50 countries and, in the last decade, it has been expanding at 8 percent/year. During 2016, 30 million tonnes of algae were harvested for direct consumption or further processing for food, pharmaceuticals, cosmetics and other purposes [2]. The brown Japanese kelp (*Laminaria*), commonly known as Kombu accounts for about 27% of world algal production for human consumption. Apart from the nutritional value (30%-45% dietary fiber, 9% protein, 35% ash and low fat content), its commercial value has been of interest to food industry and algae processing companies [3]. Nowadays, several commercial Kombu-based food products are available on the market shelves (dehydrated, powdered or canned seaweeds, crackers, pâtés and charcuterie, chocolates and food supplements). Drying is the most common preservation procedure, however, mainly in regions with large tradition of canning industries, canned algae (alone or with fish) is also available in the food market.

The aim of this work was to compare the influence of two industrial processing methods (drying and canning) with laboratorial processing (freeze-drying) on the nutritional composition of fresh edible *Laminaria.* The samples (fresh, dried, and canned) were provided from the Atlantic coastal region of Galicia and the fresh material was lyophilized in our laboratory.

The results showed a decrease of protein and fat contents when submitted to the following procedures: lyophilized (14% and 0.78% dw) > dried (10% and 0.6% dw) > canned (7% and 0.4% dw, respectively). The nutritional value loss can be due to the thermal algae processing and the nutrients dissolution in algae canning liquid. The industrial drying process is due under a temperature of 42ºC while the canning reaches sterilization temperatures (120ºC or higher). In addition, sodium chloride (which is naturally present in the samples) represents a significant percentage of algae ash in fresh weight: 28% of ash of lyophilized algae are NaCl; 67% in dried and 42% in canned algae. Washing processing of fresh seaweed with deionized water in the laboratory leads into a decrease of the salt content (expressed as NaCl).

As conclusion, the freeze-drying processing was the best to preserve all *Laminaria* nutritional value, however it's an expensive procedure for industrial application.

Acknowledgments: To ALGAS ATLÂNTICAS ALGAMAR (Pontevedra, Spain) for providing the samples. The work was supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national funds. S.M.F. Bessada and R.C. Alves thank to FCT the PhD grant (SFRH/BD/122754/2016) and the CEECIND/01120/2017 contract, respectively.

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Evolution of polyphenolic compounds during storage of hazelnuts dried under different conditions

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Prior to the consumption and/or use as ingredients in processed food, hazelnuts are subjected to different processes (drying, roasting, grinding) that can affect their chemical composition, particularly concerning taste-active compounds, such as sugars, organic acids, condensed tannins and other phenolic compounds. Among these processes, roasting has been deeply studied and it have been proved to have a detrimental impact on phenolic composition [1]; on the contrary, scientific literature is lacking about the effect of drying.

Drying is necessary to preserve chemical, nutritional and microbiological quality of hazelnuts. After harvest, fresh hazelnuts are dried to moisture levels lower than 6% wt in order to guarantee the optimal storage [2].

In the present work we assessed how different drying conditions can affect the phenolic composition of hazelnuts during a storage period up to 15 months.

Tonda Gentile Romana hazelnuts (harvest 2017) were dried in a pilot plant at three different temperatures (20, 30 and 40 °C) applying two air fluxes (v_{min}, v_{max}), until reaching a moisture content of about 6%. Hazelnuts were analysed immediately after drying (t0) and following 6, 12 and 15 months of storage at 20 °C in vacuum-sealed aluminium bags (t1, t2 and t3, respectively). Phenolic compounds were extracted from defatted hazelnuts and characterized for the total content and the antioxidant activity. The main phenolic acids and flavanols were then quantified by HPLC-DAD.

Unshelled hazelnuts were firstly characterized before the drying process. Their total polyphenolic content was 1.47±0.02 mg gallic acid eq. per gram of hazelnut (dw) and the antioxidant activity was 0.43±0.02 mg Trolox eq. per gram. Among the phenolic compounds identified by HPLC-DAD, procyanidin B1 was the most abundant (119±13 µg/g). After drying a significant decrease of the polyphenolic content and the antioxidant activity was observed in all the operative conditions considered (ca. -25%), not evidencing substantial differences depending on temperature or air flux. During storage a further decrease from t0 to t3 was observed, accounting for ca. -68% for total phenolic content and ca. -43% for the antioxidant activity. Individual phenolic compounds presented different and specific behaviours both considering drying and storage impact. In a general way, a global decrease was observed from t0 to t3, except in the case of epicatechin, which significantly increased during storage, reaching the maximum concentrations at t2.

In conclusion, these results indicate a general negative effect of drying on the hazelnut phenolic component, even when the process it performed at low temperatures. Total phenolic content and antioxidant activity generally not vary depending on the different operative conditions, but decrease during 15 month storage.

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POSTERS

Food Safety

Formation of Furfuryl Alcohol in Coffee

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Introduction:
Furfuryl alcohol is a compound that is not commonly found in foods in higher concentrations, except in roasted coffee. The reason for this single occurrence is not clearly solved but it is definitely related to the high temperatures of roasting. The temperature at which furfuryl alcohol is formed at higher concentrations is in the range of the roasting temperatures which can be up to 270 °C.

Furfuryl alcohol can be activated to a highly reactive compound by sulfotransferases in the human metabolism. This sulfated compound can react with the DNA forming adducts and induce mutations. As coffee is practically the only known commodity which contributes to the exposure the purpose of the experiments was to investigate the formation and measure the concentrations in coffee which will be a contribution to estimate the exposure to furfuryl alcohol.

Methods:
HPLC analysis is done using a C8-RP measuring the UV absorption at 217 nm eluting with a water-methanol gradient. The roasting experiments are carried out with 80 g of coffee in a Probat laboratory roaster (second crack after ca. 8 min).

Results:
In the standardized coffee brews the concentration of furfuryl alcohol is in the range of 55 to 68 µg/ml. Practically, all furfuryl alcohol is extracted during brewing and can be found in the cup. The formation kinetics show a peak quickly after the onset of roasting and after that a decrease, which can be attributed to evaporation and polymerisation. The furfuryl alcohol emission during roasting, is high (up to 57%) leading to a lower amount of furfuryl alcohol determined in samples roasted under these conditions.
The maximum concentration of furfuryl alcohol is reached faster with higher roasting temperatures. It has to be pointed out that the highest amount of furfuryl alcohol observed was at 240 °C (512 µg/g) and that the amount of furfuryl alcohol produced at 180 °C was the lowest in the experiments described here (92 µg/g).

Conclusions:
Furfuryl alcohol is formed during roasting of coffee. At present, it is not possible to reduce the amount of furfuryl alcohol in the coffee without changing the roasting conditions significantly. Coffee is a significant source for furfuryl alcohol exposure. In comparison, the concentrations found in any other foods treated at high temperatures are negligible.

Assessment of perfluoroalkyl substances (PFASs) levels in fats and oils

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Perfluoroalkyl substances (PFASs) are man-made chemicals manufactured for numerous applications. They belong to a class of compounds that tend to persist in the environment and are proposed as a new group of persistent organic pollutants. Due to the strong C-F bond and both hydrophobic and lipophilic properties they have been used in such application as cosmetics, fire-fighting foams, and water, and grease repellent coatings for fabric and food packaging. However, recent studies have documented that food and dust may be major contributors to human exposure to these compounds. Therefore, the aim of this study was to assess the levels of seven perfluoroalkyl carboxylic acids (PFCAs) and three perfluoroalkane sulfonates (PFSAs) in selected fats and oils.
Food samples, collected in Poland in 2016, including sunflower and rapeseed oils, olive oil, margarine and mix of butter and margarine were chosen because they represent the pyramid of proper nutrition. The analysis of perfluoroalkyl substances occurrence in food products represents a challenging task because of the complexity of matrices and low concentration levels expected for these compounds, so efficient preconcentration and clean-up procedures are needed. Wherefore the fats and oils sample preparation for PFCAs and PFSAs determination was conducted according to the methodology evaluated and validated in the previous study [1], based on dispersive solid phase extraction (d-SPE) followed by micro-HPLC-MS/MS. However, considering that all investigated samples contained a higle level of fat , application of additional sorbent for sample clean-up was also necessary.
Detection frequency for PFASs across the trial was 25.6%. The predominant compound in the group of PFCAs was PFOA (perfluorooctanoic acid), which was found in all analysed samples. The highest concentration for PFOA was measured in sunflower oil (1.96 ng g^{-1}). For PFSAs family most commonly detected was PFOS (perfluorooctane sulfonate), but its concentration did not exceed 0.05 ng g^{-1}. The major contributor to the total PFAS concentration in the investigated high-fat foods was PFBA (perfluorobutanoic acid), followed by PFOA. For these compounds summary concentration within the investigated group was estimated to 10.94 ng g^{-1} and 8.91 ng g^{-1} for PFBA and PFOA, respectively. Total concentrations of PFASs in particular samples ranged between 1.56 to 14.67 ng g^{-1} for margarine and sunflower oil, respectively. Sunflower oil, olive of oil, and rapeseed oil were the most contaminated food items. The Kruskal-Wallis H non-parametric test was performed to test whether there were any differences among groups (sunflower oil, rapeseed oil, olive of oil, margarine, mix of margarine and butter) for each of the 10 variables. Statistical difference in PFOA level was observed between sunflower oil and olive of oil ($p=0.0164$). No significant difference was found between the other results. As PFOA is ubiquitous in various environmental matrices, many foodstuffs derived from different origins are contaminated with this compound. High concentration of PFBA may be due to its usage as long-chain PFASs alternative. The origin and growing region may impact PFASs distribution profile and their levels in food. Having regard to the existing European standards of TWIs (tolerable weekly intakes of PFOA and PFOS set up to 6 ng kg^{-1} $week^{-1}$ and 13 ng kg^{-1} $week^{-1}$, respectively), PFASs levels seem to be worrying [2].

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Effect of ozone and sonication as an alternative to chlorine disinfection in strawberries artificially inoculated with *L. innocua*

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BACKGROUND: Despite the well-known benefits of consuming raw and minimally processed fruit and vegetables, safety is still an issue of concern. Currently, sodium hypochlorite is the most widely used disinfectant in the fruit and vegetable processing industries. However, its use has been banned in several European countries for health issues. Ozone disinfection and ultrasound processing are emerging as potential alternatives to chlorine disinfection.

OBJECTIVES: The objective of the current study was to determine the effectiveness of ozone used alone or in combination with ultrasound processing to disinfect strawberries (*Fragaria* × *ananassa*) artificially inoculated with *L. innocua*.

METHODS: Antimicrobial effects of ozone at a concentration of 0.5 ppm used alone or combined with sonication (35 kHz, 250 W, 4 ºC) were assessed *in vitro* using strawberries artificially inoculated with *L. innocua* CECT-910. The effect of processing on the native microbiota was also evaluated. Treatment time was either 2 or 5 min in both cases and results were compared to washings using tap water for 2 or 5 min and chlorine at 200 ppm (pH 6.5) for 2 min. The effect of processing on colour, texture, phenolic content, and antioxidant capacity were also studied following previously described methods both after processing and after a 6-day storage period at 5 ºC [1, 2].

RESULTS & DISCUSSION: *In vitro* trials suggested that ozone, when used alone at a concentration of 0.5 ppm did not affect the overall quality of the strawberries: no major differences were observed in the texture, colour, and other physicochemical attributes of the strawberries after processing or after storage when compared to the control (p<0.05). Similar results were observed when ozone was combined with ultrasounds at the above-mentioned conditions (p<0.05). Ozone, especially when applied for 5 min, led to a reduction in the total phenolic content of the strawberries (p<0.05). None of the studied treatments led to significant reductions in the populations of total aerobic mesophilic microorganisms or yeasts and moulds. Similar results were observed after 6 days of storage at 4 ºC. However, both ozone used alone or in combination with sonication led to 1.9- and 0,7-log ufc/g reductions in the population of *L. inocua* (p<0.05). Results were comparable to those obtained after chlorine disinfection at 200 ppm (pH 6.5) for 2 min, calculated as 2.1-log cfu/g. Moreover, ozone treatment led to significantly lower concentration of mesophilic aerobic microorganisms as well as yeasts and moulds in the washing solution, which were calculated as 1.2- and 1.2-log cfu/g after treatment using ozone and as 2.8- and 2.4-log cfu/g after washing with water for 2 min, respectively.

CONCLUSIONS: Overall, no major differences on the physicochemical quality of the strawberries were found between treatments immediately after processing and after a 6-day storage period at 5 ºC. But presence of ozone seems to reduce phenols total content after processing. Ozone and sonication did not affect the native microbiota of the strawberries, although a lower content of microorganisms was observed in the washing water when compared to the control. However, ozone and sonication did reduce the content of *L. inocua* suggesting that these technologies could be used to produce safer products. Further studies are needed to fully understand the potential of ozone and sonication to inactivate pathogenic microorganisms.

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Study on the Migration of Strawberry and Grape Plants to Heavy Metal Enrichment Ability

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In order to find out the migration and enrichment of heavy metals in plants, two different plants strawberry and grape were selected as testing sample. Collect strawberry fruit, plant and in situ soil samples in the main strawberry producing area. Meanwhile, the contents of 8 heavy metals such as lead(Pb), cadmium(Cd), chromium(Cr), nickel(Ni), copper(Cu), zinc(Zn), mercury(Hg) and arsenic(As) were determined by taking 11 viticultural cultivars as testing subjects, collecting soil samples and plant organs at the same time during maturity.

This indicated the migration and enrichment ability of heavy metals in the soil-strawberry system: The enrichment coefficients of heavy metals in different organs of strawberry varied significantly, which showed different trends in enrichment (Using F to represent enrichment coefficients). The sequence among cadmium(Cd), chromium(Cr), nickel(Ni), and arsenic(As) from high to low is that F-root, F-stem, F-leaf, F-fruit; While among copper(Cu), zinc(Zn), mercury(Hg) and lead(Pb), the sequence from high to low is F-root, F-leaf, F-stem, F-fruit. In addition, the enrichment ability of strawberry to heavy metals cadmium(Cd) was greater than that of copper(Cu), also zinc(Zn) was greater than nickel(Ni) and mercury(Hg), and chromium(Cr) was greater than lead(Pb) and arsenic(As). Furthermore, the transmission capacity of heavy metals from roots to aboveground parts organ indicated that mercury(Hg) was higher than nickel(Ni) and arsenic(As), and zinc(Zn) was higher than copper(Cu), lead(Pb), chromium(Cr) and cadmium(Cd).

The enrichment ability and migration characteristics of heavy metals in soil-grape system are basically clear. Grape plants differ significantly in their ability to enrich different heavy metals in soil, which can be divided into three categories: The first type is cadmium(Cd), which has strong enrichment ability; The second type is zinc(Zn), which has a certain enrichment capacity; The third is chromium(Cr), lead(Pb), nickel(Ni), mercury(Hg), arsenic(As), and copper(Cu), which has no enrichment. Different heavy metals have different migration characteristics in the soil-grape system. The migration of zinc(Zn) in the root-soil interface zone, root and stem, and stem-leaf interleaf was relatively smooth, while the migration of copper(Cu) between root-stem and stem-leaf was basically smooth. The transmission of mercury(Hg) between stems and leaves was extremely smooth, while the transmission of chromium(Cr), lead(Pb), arsenic(As), nickel(Ni) between stems and leaves was relatively smooth, and cadmium(Cd) had a significant enrichment effect. The absorption ability of heavy metals was obviously different between different grape varieties.

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Monitoring of ochratoxin A in cocoa from the southern region of Bahia / Brazil.

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The mycotoxin monitoring is essential for us to have healthy food and contamination free. Brazil is the seventh greatest cocoa producer, after Ivory Coast, Ghana, Indonesia, Ecuador, Cameroon and Nigeria. The state of Bahia, stands out for being the largest producer, with about 48% of the total production.

The ochratoxin A (OTA) is mostly produced, at tropical weather, by *Aspergillus ochraceus* and *Aspergillus* from Nigri's group, including *Aspergillus carbonarius.* Classified at 2B group (possible human carcinogen), according to IARC (IARC, 1993). OTA has been reported in cocoa powder native of Ivory Coast, Nigeria and Cameroon (BONVEHI, 2004); sold at Italian stores (TAFURI *et al.,* 2004) and at Brazilian cocoa beans (COPETTI *et al.*, 2010; MAGALHÃES *et al*, 2011).

Ochratoxin A contamination was determined in samples from 4 consecutive crops (2015. 2016, 2017 e 2018) developed in the south of Bahia, by ultra-high performance liquid chromatography with fluorescence detector (UPLC-FLD) (Limit of detection (LOD): 0,900 $\mu g.kg^{-1}$; Limit of quantitation (LOQ): 2,500 $\mu g.kg^{-1}$; Recovery: 89,4 $\mu g.kg^{-1}$ for OTA) (MACIEL, 2017). The results were presented according to current legislation and since 2016, 2017 and 2018 crops (harvest), OTA was not detected at cocoa samples from the south of Bahia.

Table 1: Ochratoxin A detection on the analyzed cocoa crops

Crop (Harvest)	Ochratoxin A $\mu g.kg^{-1}$	Means OTA $\mu g.kg^{-1}$
2015	<LOD-8.200	1.366
2016	<LOD	<LOD
2017	<LOD	<LOD
2018	<LOD	<LOD

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Comparative digestomics of Tropomyosin of vertebrates and invertebrates in real food matrix

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Shellfish, is a highly nutritive food resource in the world, but also among the eight allergic food groups accounting for approximately 90% of all immunoglobulin E food allergies worldwide [1]. This work focuses on the only well-recognized major allergen muscle protein tropomyosin(TM) that is responsible for cross reactivity between shellfish and other invertebrates [2]. By contrary, TM of vertebrates (chicken, pig, cow) is not a prominent allergen. The stability of food allergens to digestion is an important factor contributing to their allergenicity. Most in vitro digestibility studies are based on the protein extract rather than whole food matrix thus overlooking its effect on TM stability [3]. Our objective was to primarily test the pepsin digestibility of invertebrates and vertebrates (raw and thermally treated based on their real life consumption modes) mimicking the gastric digestion under standardized conditions. To closely observe and compare the vertebrates' and invertebrates' TM stability, we aimed to perform the specific antibody based western blot analysis with two primary antibodies; ❶Rabbit anti shrimp TM antibody (invertebrates), and ❷ Rabbit anti human TM antibody (species reactivity to vertebrates).

Methods: Thermal treatment of selected samples to compare TM heat stability, Standardized static in vitro methods of simulated gastric digestion[4] for the evaluation and comparison of TM resistance to pepsin, Sodium Dodecyl Sulfate-Polyacryl amide Gel Electrophoresis (SDS-PAGE) of digesta supernatant under reducing and non-reducing conditions to quantify proteins and compare thermally treated invertebrates and vertebrates protein profiles focusing on TM, specific antibody based semi dry Western blot analysis.

Results and discussions: SDS-PAGE analysis of vertebrates and invertebrates' samples showed a range of proteins in varied amounts between 10-250 kDa. Depending upon samples, varied numbers of prominent protein bands were observed including the distinct bands corresponding with the molecular weights of TM(37-39kDa). In agreement with publications, TM was, indeed, resistant against pepsin digestion as well as thermal treatment prominently in case of invertebrates. This was confirmed upon Ab based Western blot analysis. Our results show that, upon thermal treatment, TM is partially degraded as is observed in case of raw and cooked beef electrophoretic profile as well as WB analysis. Significantly, upon pepsin digestion, TM (allergen) is completely degraded in vertebrates in contrast to the invertebrates' TM (which is pepsin resistant and heat stable).

This result provides an insight on the differences in digestibility of allergenic versus non-allergenic TM in real food matrix and upon thermal treatments of solid food samples.

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Multidetection of Antibiotics in piglet liver by Ultra-High-Pressure-Liquid-Chromatography tandem Time-of-flight Mass Spectrometry

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Antibiotics may be administered in food-producing animals for therapeutic and prophylactic purpose. The use of antibiotics as growth promoters in food-producing animals can also be considered, although fraudulent in Europe because it can lead to residues of these compounds in edible matrices. [1] The persistent consumption of animal foods containing antibiotic residues can cause to direct toxic effects, such as allergic reactions in some hypersensitive individuals, or indirect effects, such as appearance of bacterial strains resistant to the drugs used in veterinary medicine, becoming a worldwide concern. [2] Therefore, it is of extreme importance for public health, as well as consumer food safety, to monitor possible antibiotics that can be found in animals for food consumption, in particular piglets since it produces a highly consumed meat in Portugal.

The presence of residues in products of animal origin is a matter of public health concern. Therefore, the European Commission has determined the need for the compulsory control of veterinary medicinal products in food of animal origin, which is designated as a veterinary medicinal product for human consumption. Consequently, maximum residue limits (MRLs) have been established in foodstuffs of animal origin according to Regulation (EU) No. 37/2010 [3].

In this study, 21 piglet livers sample were analyzed using an ultra-hight-pressure-liquid-chromatography-tandem time-of-flight mass spectrometry (UHPLC-ToF-MS) to detect the presence of 45 antibiotics from 7 different families such as tetracyclines, sulphonamides, quinolones, penicillin's, macrolides, cephalosporins and trimethoprim. Antibiotic residues were determined to be around 47% in the twenty-one samples analyzed. Ten of the samples did not contain any type of antibiotic, seven samples contained one antibiotic and four samples contained two antibiotics. The antibiotics detected in the examined samples were enrofloxacin, doxycycline, ciprofloxacin and oxolinic acid. All these antibiotics were found in minimal concentrations, not exceeding the MRL.

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Effect of Ozone and Electron Beam Irradiation on Degradation of Zearalenone and Ochratoxin A

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Zearalenone (ZEN) and ochratoxin A (OTA) are the key concerns of the food industry for its toxicity and pollution scope. In this study, effects of ozone and electron beam irradiation (EBI) on degradation of ZEN and OTA were investigated. Results demonstrated that 2 mL of 50 μg/mL ZEN was degraded completely after 10 s of treatment by 2.0 mg/L ozone. The degradation rate of 1 μg/mL ZEN by 16 kGy EBI was 92.76%. Methanol was superior to acetonitrile for degradation of ZEN. The degradation rate of 2 mL of 5 μg/mL OTA by 50 mg/L ozone at 180 s was 34% and of 1 μg/mL OTA by 16 kGy EBI exceeded 90%. Moreover, OTA degraded more rapidly in acetonitrile. Ozone performed better in the degradation of ZEN, whereas EBI was better for OTA. The conclusions provided theoretical references and practical basis for the degradation of different fungal toxins.

Keywords: Ozone; Electron beam irradiation; Degradation; Zearalenone; Ochratoxin A

Can acrylamide influence the viability of lactic acid bacteria?

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Background. A lot of studies have already proved that acrylamide (AA) can exert genotoxic and cytotoxic impact on human organism. There are studies demonstrating that after introduction of 1%–3% acrylamide into the growth medium *Escherichia coli* cells undergo the various changes, such as blockage of cell division, elongation of cells, inhibition of DNA synthesis, decrease in osmotic stability and ultrastructural alterations of the outer membrane [1]. *E. coli* together with other microorganisms that are members of human intestine microbiota and colonize intestine can be exposed on acrylamide after intake of various fried, grilled, toasted, roasted or baked foodstuffs such as roasted potatoes and root vegetables, chips, crisps, toasts, cakes, biscuits, cereals and coffee. Lactic acid bacteria (LAB) constitute very part member of intestinal microbiota and play beneficial role in our organism.

Objectives. Therefore, the aim of this study was to evaluate whether acrylamide can influence the viability of lactic acid bacteria.

Material and methods. Microorganisms used in the study were lactic acid bacteria: *Lactobacillus brevis* (DSMZ 20054) and *L. plantarum* (DSMZ 20205) purchased from Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zelkulturen GmbH (Braunschweig, Germany),as well as probiotic strain *Lactobacillus acidophilus* LA-5 /LA5/ (Christian Hansen, Hørsolm, Denmark). Experiments were carried out in model solutions (0.45% NaCl and 0.45% bacteriological peptone) with the addition of various acrylamide doses (0-100 µg/ml). Solutions were inoculated with bacteria and incubated for 48 h in 37°C. The percentage of live, injured and dead bacteria was assessed by flow cytometry (BD Accuri™ C6 Flow Cytometer) using BD™ Cell Viability Kit at 0h and after 24 h and 48 h of incubation. All experiments were carried out in five replicates and results were expressed as means ± standard deviations. Statistical analysis was carried out in R, version 3.3.3 (2017-03-06) -"Another Canoe", copyright (C) 2017 The R Foundation for Statistical Computing.

Results. Our results demonstrated that acrylamide can influence the viability of lactic acid bacteria, but its impact depends both on the AA concentration and on the bacteria species. *L. acidophilus* LA-5 was more sensitive to the AA presence, while *L. brevis* was the less sensitive. The statistically significant differences were related mainly with the percentage of injured and dead cells. Moreover, in case of *L. plantarum* we observed that acrylamide (especially in concentration of 30 µg/ml) strongly influenced the morphology of bacteria. Based on the fact that in the population appeared cells with both a 2-fold and several times stronger FL1 fluorescence signal (thiazole orange), we conclude that acrylamide stimulated the division of *L. plantarum* and cells in the form of diplobacillus and streptobacillus were present in the population. This has been confirmed in microscopic preparations.

Conclusion. We conclude that acrylamide present in food can modulate the viability of lactic acid bacteria and therefore can influence their activity in food products or, after colonization, in human intestine.

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Aluminium in infant cereal: total concentration and bioaccessible fraction

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Infant cereals have become an excellent alternative as a complementary food for children, as they are produced from corn, rice, wheat and oat flour, and are usually enriched with specific nutrients. This requires consideration of the correct nutrient balance (proteins, carbohydrates, fats, minerals and vitamins), quality of essential fatty acids, occurrence of potentially harmful substances and the correct introduction of potential allergic ingredients (Pehrsson et al., 2014). High levels of inorganic contaminants, such as aluminium (Al), can be found in these foods, which could represent a risk to health. Al is the most abundant metallic element and can be found in several foods, food ingredients and materials such as food additives, baking powder, processed cheese, meat products, and cooking and storage utensils in the food industry (Saracoglu et al., 2007). In terms of toxicology, Al can act as a potent neurotoxin that causes impaired mental development in the long term exposure and can also be incorporated into infant bones, resulting in a weakened bone structure. Infants have been reported to be highly vulnerable to Al exposure and have a narrow tolerance to this non-essential element (Ahmed et al., 2016). Therefore, based on the relevant concern about Al toxicity, the objectives of this work were: to determine the levels of Al in 9 different samples of infant cereals (multicereal composition) from 3 distinct batches and 3 different brands commercialized in Brazil, and study the bioaccessible fraction of Al. Total Al was determined by acid-microwave digestion and ICP OES, and an *in vitro* digestion method was employed to evaluate the bioaccessibility. Multicerals composition presented high total Al concentrations with mean values of 3.46 mg kg^{-1} for brand A, 7.13 mg kg^{-1} for Brand B and 6.59 mg kg^{-1} for brand C. Regarding Al bioaccessible fraction, mean values of 3.5%, 3.6% and 6% were found for brand A, B and C, respectively. In general, despite high obtained values of total Al in cereal sample, its bioaccessible fraction is relatively low; moreover, further studies must be developed regarding Al bioaccessibility considering the ingredients contribution of this food matrix for infants' diet.

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Proposal of health risk assessment of combined exposure to Se, Hg, and iAs

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Selenium is an essential trace element and cereals and seafood are the main Se source. The Se status in the European population is considered as suboptimal. European soils poor in Se hamper adequate intake of Se via cereals [1]. Moreover, seafood, cereals, and even mushrooms - besides of being good sources of Se - may contain significant amounts of toxic elements. Despite the high bio-availability of Se in seafood, studies that were conducted to improve Se levels by increasing seafood intake, gave positive results. However, they did not meet the expectations [2]. The interactions between Se and pollutants such as Hg and iAs reduce the availability of Se [2,3]. Hg shows extremely high affinity to Se leading to formation of inert complexes Hg-Se [4]. Furthermore, in mammals, studies showed an effective joined equimolar co-excretion of iAs and Se that is due to the formation of $[(GS)_2AsSe]^-$ [5]. Thus, decreases in efficiency of dietary Se intake can be explained by these interactions. Yet, it is also worth considering that Se is capable of antagonizing harmful effects of Hg and iAs [4,5].

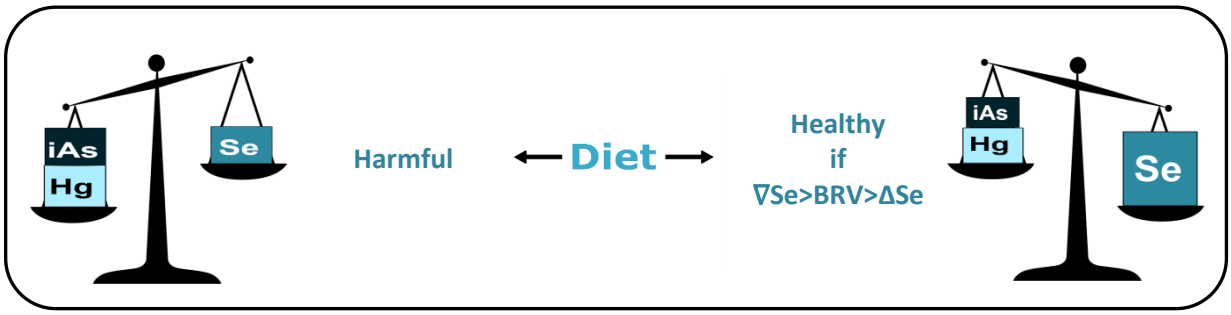
Therefore, an adequate dietary surplus of Se with respect to these toxic elements is mandatory to guarantee essential Se-dependent functions, such as the activity of selenoenzymes.

The Beneficial Risk Value (BRV) is a criterion of risk assessment [3] based on the aforementioned Se-Hg interaction. BRV compares potential daily intake (PDI) of Hg and Se. Considering the losses of Se due to the molar amounts of iAs, a novel BRV could be calculated by the formula:

$$BRV = PDI_{Se} - \Delta Se - (PDI_{Hg} + PDI_{iAs})$$

This index considers unsafe BRV < 0 (possible adverse effects of Hg and/or iAs). BRV > 0 indicates a surplus of Se, in which case it might be considered healthy if the intake exceeds the minimum requirement (ΔSe) without reaching its threshold of toxicity (∇Se).

Components of the diet containing these elements are mushrooms, seafood or rice. Likewise, drinking water is the main source of iAs in certain populations. In conclusion, it is advisable to evaluate the combined exposure to Hg, iAs and Se in order to determine the Se status more accurately and to assess if detrimental effects of Hg and iAs might be avoided.



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Autolytic changes involving proteolytic enzymes on Atlantic salmon (*Salmo salar*) preserved by hyperbaric storage

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The concept of hyperbaric storage (HS) at low and room temperatures has been studied as an opportunity for fish preservation by several authors lately [1, 2, 3, 4], showing the efficiency of HS in extending the shelf-life of fresh fish. However, as the authors are aware, the effect of HS on the enzymes activity from fresh fish is inexistent.

So, the effect of HS (50, 60 and 75 MPa) at low/room temperature (10, 25 and 37 ºC) on proteolytic enzymes and muscle proteins stability of Atlantic salmon (*Salmo salar*) was assessed. Different storage pressure/temperature conditions were tested, and storage experiments were performed for 10 days. The results were compared to control samples stored at atmospheric pressure (AP, 0.1 MPa) at the same storage temperatures (10, 25 and 37 ºC) and under conventional refrigeration (5 ºC) during the same time. Enzymatic activities of lysosomal (phosphatase acid, cathepsins B and D) and cytosolic (calpains) proteases, myofibrillar fragmentation index (MFI) and sarcoplasmic protein content were evaluated.

Generally, residual activities of acid phosphatase, cathepsin B and D, and calpains decreased during storage when compared to fresh salmon, with a pronounced effect being observed for storage at 37 ºC (in both HS and AP samples). A pronounced increase of MFI was observed at 75 MPa (25 and 37 ºC) after 10 days (3.2- and 4.3-fold, respectively). On the other hand, HS at 60 MPa/10 ºC showed a decrease of MFI values (45%), being not significantly different to samples stored at AP/5 ºC. Similar results were observed at 60 MPa/25 ºC (71%). For sarcoplasmic proteins, at 60 MPa (10 and 25 ºC), there were no significant differences on sarcoplasmic proteins content. However, at 75 MPa/25 ºC, sarcoplasmic proteins only decreased after 10 days (39%), while at 75 MPa/37 ºC, the pressure effect was more immediately detected, with a reduction to 58% after 3 days, with no further statistical differences during the 10 days (67%) and no statistical differences being observed also compared to respective control samples at AP/37 ºC.

So, by increasing storage temperature, a considerable effect on proteolytic activity was observed during HS. However, at low temperatures/pressure, muscles proteins seemed to be less affected.

Acknowledgments: Thanks are due to the University of Aveiro and FCT/MCT for the financial support for the QOPNA research Unit (FCT UID/QUI/00062/2019) through national founds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement and for supporting the PhD grant of Liliana G. Fidalgo (SFRH/BD/96984/2013).

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Quantification with reference material – is this reliable?

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Sensitive, fast, and inexpensive analytical techniques are necessary to supervise the composition of processed food. Additionally, it is important to differentiate between contamination and intentional addition. Therefore, quantitative methods are mandatory.

One such method is quantitative real-time polymerase chain reaction (qPCR). By means of amplification of species specific DNA sequences, even traces of DNA can be detected. And as DNA is relatively heat stable[1], this method can be applied for processed food. When probes with different fluorochromes are used, several DNA sources can be simultaneously detected (multiplex qPCR).

The main problem is quantification. An easy and widely used solution is to apply a serial dilution of the target DNA. However, the results are given in copies of DNA and therefore, no direct conclusion about the actual added amount of target material is possible. Another approach is to produce reference material containing the target material in different concentrations within the detection range. The thereof isolated DNA samples are then used as a straight calibration line. In this way the results are presented in g of added target per kg food product and the direct conclusion of how much target was added is possible.

The aim of the presented study was to validate the reliability of quantification via reference material. For this purpose, emulsified type sausages were chosen as a model for processed food and the six main cereal species as target DNAs. And to be fast and inexpensive, two triplex qPCR systems (barley, oat, and rye and maize, rice, and wheat) were developed. Emulsified type sausages were produced and spiked with 0.0005-0.1% plant protein per species and produced at different conditions. As production parameters, post-processing (none vs. grilling vs. storing), packaging (artificial casings vs. cans), and production temperature (75°C vs. 121°C) were investigated. For the quantification analysis, emulsified type sausages produced at 117°C in cans and without any post-processing were used. In the last step, the production of reference material was validated at low and medium temperatures for three concentrations.

While the post-processing and packaging parameters had no influence on the detectability of plant DNA in meat products, differences were seen for the production temperature. The recovery rate varied between 161±64% - 260±71% at low and between 62±11% - 95±22% at high production temperature in comparison to the reference sausages. Therefore, the production temperature had to be checked only for the validation of the production of reference material. At low production temperature, the values for the coefficient of variation (CV) varied between 19.5-39.0% (0.001% plant protein), 11.0-28.1% (0.01% plant protein), and 7.8-18.1% (0.1% plant protein). At medium production temperature, the CV values were 18.1-40.3% (0.001% plant protein), 29.9-44.7% (0.01% plant protein), and 17.3-28.9% (0.1% plant protein). Hence, the accuracy was satisfactory at 0.01% and 0.1% plant protein at low temperature and at 0.1% plant protein at medium temperature (CV ≤ 30%). At all other conditions, the accuracy was not given (CV ≥ 30%)[2].

All in all, the quantification with reference material is possible. However, as the measurement uncertainty is quite high and dependent on the production temperature, it is neither reliable nor simple. Therefore, either the reference material has to be produced at the same production temperature as the sample or other analytical methods for quantification have to be developed.

Acknowledgments: The authors thank Tina Schobert for her technical assistance in the laboratory and Siegmar Eckl for producing the emulsified type sausages.

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Assessment of Table Grape Quality Levels by using Headspace Solid Phase Microextraction Mass spectrometry-based Electronic Nose (HS-SPME MS-eNose) and ^{1}H Nuclear Magnetic Resonance spectroscopy (^{1}H NMR) fingerprinting techniques in combination with Multivariate Statistical Analyses

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Introduction:
The Quality Level (QL) of table grape is defined through sensory evaluation of the its overall appearance by a five-point rating scale (from 5: excellent to 1: extremely poor). Since this evaluation is dependent on subjective judgments, the aim of this work was to develop methods based on MS-eNose and ^{1}H NMR data for an objective QL assessment of two table grape cultivars, i.e. Vittoria and Italia.

Methods:
Table grape bunches were stored at 5 and 10 °C and sampled after a number of days needed to reach each QL. Berries were homogenized, centrifuged and the supernatant was analyzed by HS-SPME MS-eNose and ^{1}H NMR. LDA and PLS-DA were applied on data to discriminate samples based on the five QLs (five class discrimination) and on their marketability/non-marketability (two class discrimination).

Results:
The model performances were expressed in terms of the prediction ability calculated by V-fold cross-validation procedure (CV=20%). For both cultivar, NMR model average prediction abilities ranged from 92 to 93%, and from 76 to 79%, in the two and five class discrimination, respectively. Better results were obtained in case of the MS-eNose models with mean prediction abilities ranged from 98 to 100% (two class discrimination), and from 86 to 100% (five class discrimination). In particular, the best prediction abilities of 100% were obtained in case of *cv.* Vittoria for MS-eNose PCA-LDA in both discriminations (two and five class discriminations) and for MS-eNose PLS-DA in the two class discrimination.

Conclusions:
Taking into account the results obtained, both analytical technologies adopted herein could be used as valid and rapid tools for the table grape quality evaluation. In details, although NMR data can provides additional information on the identification of quality marker metabolites MS-eNose predictive models showed better performances in both types of discriminations.

Ensuring food safety and satisfaction for the celiac patients

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For consumers following gluten-free diet it is important to pay close attention to a safe and gluten-free foodstuffs, thus a reliable measurement method should be available as well as authentic label information must be provided. The aim of this paper is to present the main milestones of the gluten detection and to demonstrate how the current regulatory system contributes to consumer safety and satisfaction. Wheat and cereals containing gluten are widely used ingredients in the food industry. It may be present in several foodstuffs that lay consumers would not expect. It can be a flavour enhancer or texturizer in various food products (e.g. meats or confectionery), and on the other hand, a gluten-free food may contaminate accidentally with celiac active cereals during harvesting, transport, storage or processing.

There are several methods for detecting and analyzing gluten (including microscopy, electrophoresis, dieters chromatography, immunology or DNA-based methods, etc.), but currently, according to CODEX STAN 118-1979, the quantitative detection of gluten has to be primarily protein-based, that is, an immunological method (R5-ELISA) **[1]**. If there is a method with the same sensitivity and specificity as the immunological method for the quantitative analysis of raw and processed, heat-treated foods, it also could be a possible way of analysis.

Cereal researchers involved in this topic continuously strive to develop detection methods, which are more sensitive and more specific than current ones. Initially, they concentrated on the development of antibodies that recognize gliadin, then the focus shifted to the development of antibodies that recognize the T-cell stimulating epitopes of gliadin that trigger celiac disease. As an alternative method, the most accepted technique is DNA-based PCR detection **[2]**.

In addition to the continually refining detection methods, the regulatory environment (828/2014/EU, 1169/2011/EU) also contributes to consumer safety **[3] [4]**. Based on our questionnaire survey among Hungarian consumers, regulations have received positive feedback. Since the widespread use of trade mark registered by the Association of European Coeliac Societies (AOES) and the mandatory labelling of substances or products causing allergies or intolerances, consumers having diet have become more informed and safe.

Continuous improvement of detection methods and the application of appropriate methods, as well as authentic labelling information are essential elements in enhancing the safety and satisfaction of consumers having celiac disease.

Acknowledgments: The authors would like to thank the project titled “Gluten-free egg replacement additives providing texture identical to that and plant-based products intended to use them” with registration no. TÉT_15_IL-1-2016-0019, realized through a Hungarian-Israeli scientific and technological cooperation supported by the NKFI Office.

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Country of origin as an indicator of food safety - the legislative framework and consumer perception

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According to European regulations, the indication of the country of origin or place of provenance of the food is obligatory, where its omission could mislead the consumer of true country of origin or place of provenance **[1].** The indication of origin is currently mandatory for some foods groups, such as beef and beef products, honey, fruit and vegetables, fish and olive oil, and additionally when food declaration has statements, pictures, terms or symbols of some specific country. The country of origin or place of provenance of the primary ingredient have to be indicated when it is different from the country of origin of the final products. New EU Regulation, which enters into force in April 2020, prescribes the manner of declarations of the primary ingredient origin **[2]**. Voluntary indications such as geographical statements included in, or accompanying the name of the food, may also be part of product designations protected as geographical indications according to specific EU legislations.

A country of origin is often perceived by consumers as a product attribute, affecting their perception of product quality and safety. The influence of the country of origin on consumer's perception of product safety depends to their knowledge of the country and, in certain cases, the level of economic development of the country **[3]**. Many consumers will rather choose products manufactured locally or products produced in countries with cultural, political and economic similarities with their homeland **[4]**. Researcher have shown that the country of origin of food can automatically influence costumers evaluations and behavioural intentions.

Therefore, a country of origin a signal to predict food quality prior to purchase for consumers with limited prior experience and when facing increasing insecurity about food safety. Consumer education and understanding of labelling of food origin will help consumers to make the right purchasing decisions that will be a guarantee of food quality.

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Development of a new method for the determination of triphenylmethane and tiazine dyes in fish muscle

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Malachite green (MG), crystal violet (CV), brilliant green (BG) and their metabolite leucomalachite green (LMG) and leucocrystal violet (LCV) belong to the triphenylmethane family of dye. Methylene blue (MB) is one of the thiazine dyes; its metabolites are probably its derivatives such as azur A (AZA), azur B (AZB), azur C (AZC) and thionine (TH). MG, CV, BG and MB are pharmaceutically active dyes used as a fungicide and ectoparasiticide in fish [1]. Due to demonstrated toxicity in tests on laboratory animals, their use in farmed fish intended for consumption by humans is not allowed [2]. Thus the presence of their residues in fish muscles after potential illegal use should be controlled using appropriate analytical methods [3]. For that reason we have developed a new method for the determination of MG, CV, BG, LMG, LCV, MB, AZA, AZB, AZC and TH in fish muscle using LC-MS/MS.

The developed method consists of an acetonitrile extraction of the dyes from fish muscles followed by centrifugation. Next, the supernatant is mixed with alumina oxide, centrifuged and cleaned up on a SPE with PSA. The obtained eluate is injected on a LC-MS/MS system with a positive electrospray ionization operated in a multiple reaction monitoring (MRM) mode. The separation of analytes in the fish muscle extract is performed on a phenyl-hexyl analytical column with acetonitrile and ammonium acetate buffer (pH 3.5) used as mobile phases with a gradient elution.

Finally, the whole method has been successfully validated aaccording to the guidelines laid down by the European Decision 657/2002/EC [4] and all validation criteria have been in the required ranges. The decision limits have been in the ranges from 0.25 to 0.5 µg/kg for all tested dyes. The recoveries have varied between 82% and 114% while the coefficient of variation of the method has been less than 19 % under within-laboratory reproducibility conditions.

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Effect of starter cultures in six biogenic amine content of traditional Portuguese sausages

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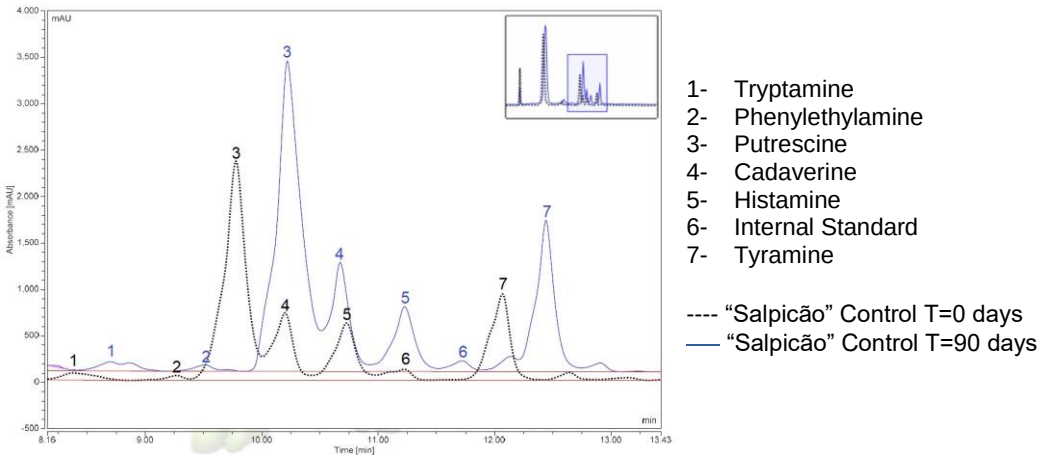
This study aims to study the effect of the addition of a LAB strain, known to be active against some pathogens that can be found in cured meat products, on the levels of six biogenic amines in traditional Portuguese sausages during storage.

Samples of traditional Portuguese sausages like “Alheira” and “Salpicão” were studied for the presence of six biogenic amines. Tryptamine, phenylethylamine, putrescine, cadaverine, histamine and tyramine concentrations were determined during storage time of 90 days for “Alheira” and 180 days for “Salpicão” at intervals of 15 days. Similar products were produced with the addition of starter culture (LAB samples) and without, here called control samples. All samples were vacuum packed and stored at 4˚C. Amines were extracted and derivatized according to J. AOAC International method (AOAC, 1999). Quantification was obtained by HPLC. A paired t-test was performed for each parameter (tryptamine, phenylethylamine, putrescine, cadaverine, histamine and tyramine) and for each product (“Alheira” and “Salpicão”) over the study time using Microsoft Excel 2016. In this analysis it was used the average of two measurements.
The study of “Alheira” product showed significant differences in 2-phenylethylamine concentration ($p<0.001$) with lower value in control samples, and in cadaverine ($p=0,021$) and tyramine ($p<=0.008$) with lower values in LAB samples.
The study of “Salpicão” product showed significant differences in tryptamine concentration ($p<0.001$) and histamine ($p=0.031$) with lower values in LAB samples; 2-phenylethylamine ($p=0.012$) and cadaverine ($p=0.046$) with lower values in control samples.
Despite of that, overall, it can be concluded that the inclusion of the LAB starter does not affect the intrinsic quality features of the studied samples, the determined biogenic amine values in all samples being according to literature values found in this type of products.

Fig.1. Two chromatograms of six amines in “Salpicão” control samples in zero and 90 days storage.



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POSTERS

Food Sustainability

Protein quantitation in fish hydrolysates - a feasibility study into the potential of ^{1}H-NMR

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Enzymatic degradation by proteases is a key technology in the utilization of fish rest raw materials. The quantitation of protein concentration, especially in the soluble phase, is of prime importance both in the process development phase and for process control during the production of protein hydrolysates. Speed, accuracy and trueness are key requirements for a successful method for these purposes. NMR is one of the most promising analytical technologies currently available regarding analytic power, speed and potential for online implementation. Therefore, the aim of this work was to investigate the potential for applying ^{1}H-NMR for direct quantitation of protein or/and amino acids in crude hydrolysates.

As an initial model for carp rest raw materials, pieces of filet meat from carp was used. The approach was to produce an array of hydrolysates, spanning a variety of enzymes and hydrolysis reaction times, subjecting the soluble phase of hydrolysates, alongside relevant standard samples, to a wide set of analytical methods.

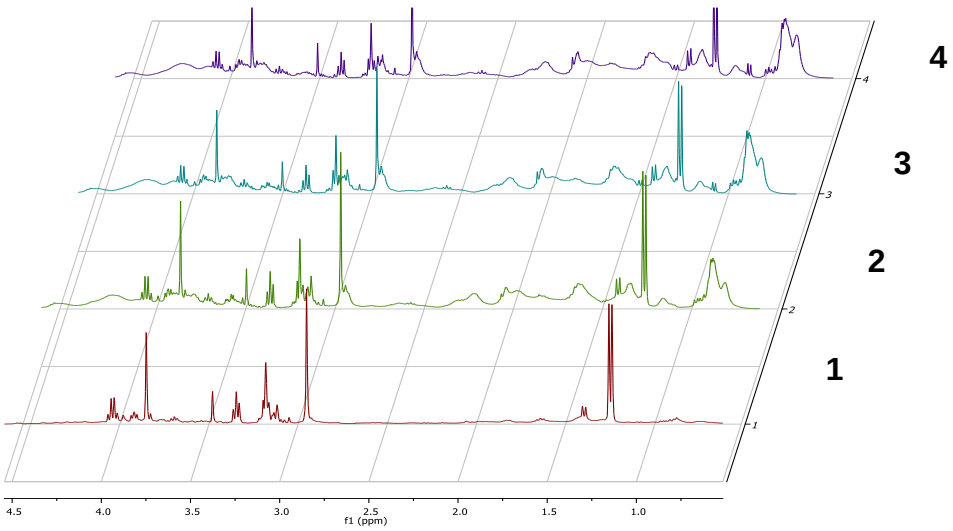

Figure 2. Area 0-4.5 ppm of the ^{1}H-NMR spectra of carp proteins solubilized after enzyme treatment (2-4) or without enzyme treatment (1)

Extra Virgin Oil's quality parameters by sustainable methods

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Europe is the world's largest producer of extra virgin olive oil (EVOO) with 2,317,174 tons in 2014. Spain, Italy and Greece cover more than 90% of European EVOO production. Extra virgin olive oil is a high value product, obtained from the mechanical pressing of olives. Several studies correlate the health benefits due to its composition: high content of unsaturated fatty acids (more than 75%, especially mono-unsaturated oleic acid), presence of phytosterols and antioxidant compounds.
The economic implication of this commodity makes it particularly susceptible to fraud. The COMMISSION REGULATION (EEC) No 2568/91 sets out several "characteristics of olive oil and olive-residue oil and on the relevant methods of analysis" in order to guarantee the purity and quality of the oil. The legislation's annexes illustrate the analysis methods and the decision tree "for verifying whether an olive oil sample is consistent with the category declared" (i.e. free fatty acidity ≤ 0.8 % for extra virgin olive oil). These methods take into account the variety of each matrix and define the amount of sample and reagents that have to been used for the determination of the quality parameters.

The aim of this work is to develop optimized methods (starting from the official ones) and make them sustainable by using a smaller amount of chemicals (e.g. reagents, solvents), reducing also the production of waste and the sample needed. Since the production and disposal of solvents has negative effect on the environment, the evaluation of sustainable methods is a very topical issue. The quality parameters chosen are free fatty acidity, peroxides number and anisidine number (from Regulation EEC No 2568/91 and ISO 6885: 2016).

A comparison between the regulation's method and the new ones was made. The new methods were validated and their results were not statistically different ($p<0.05$) from the standard ones. The miniaturized methods have greater sustainability, saving more than 45% of resources (fig. 1).

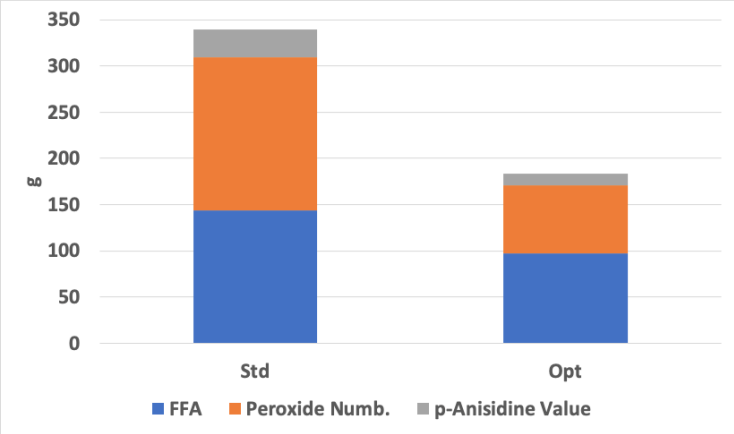

Fig.1. Total waste weight in standard and optimized methods.

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Valorisation of dried horseradish press cakes as a potential for value-added products

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Horseradish processing into juice results in large amount of press cakes. Horseradish roots and leaves are rich in different biologically active compounds, for example phenolic compounds, sulfur-containing volatile compounds and dietary fibre. After juice extraction, many valuable compounds remain in the press cakes.
The aims of this research was to evaluate the potential of differently dried horseradish roots and leaves press cakes for value-added products.
There were used different drying methods (freez-drying, microwave-vacuum drying and convection drying at 40, 60 and 80 °C) for preserve biologically active compounds of horseradish roots and leaves press cakes. The fresh horseradish roots and leaves press cake were used as control. Two steps ultrasound-assisted extraction (UAE) with acetone and ethanol / water (80/20 by volume) at room temperature was performed. The total phenol (TPC), total flavonoid (TFC), total flavonol, total flavan-3-ols and total phenolic acid content and individual profile by high performance liquid chromatography (HPLC) of the horseradish roots and leaves press cakes extracts was measured. As well as antioxidant activity was measured on the basis of scavenging activities of the stable $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals and reducing power. Additionally volatile compounds content was measured.
Results showed that content of phenolic compounds and their antioxidant activity in tested samples significantly ($p<0.05$) differed between used drying method. For preserve of total phenolic, flavonoid, phenolic acid the best drying method was freez-drying in both cases (horseradish roots and leaves press cakes). The main individual phenolic compounds in the horseradish roots and leaves press cakes were kaempferol and rutin. But microwave-vacuum drying and convection drying at 80 °C was the best drying method for preserving of antioxidant activity. Comparing convection drying at different temperatures, the higher content of biologically active compounds was detected in the samples using 80 °C drying temperature. The main volatile compound in drying horseradish press cakes were acetic acid, but allyl isothiocianate were detected almost exclusively in the fresh horseradish roots and leaves press cakes.

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Characterisation of lipophilic extracts from pine nut skin

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Stone pine (*Pinus pinea* L.) is widely present in the Mediterranean influence region and is well-known mainly for the economic value of its edible pine nuts. Pine nut skin, resultant of the pine nut processing, represents about 2.4% of the whole kernel weight [1]. Thus, the annual volume of this by-product is estimated approximately as 550 Mt worldwide [2]. Although nuts skins have been demonstrated as valuable sources of phytochemicals with health beneficial effects [3,4], pine nut skin composition is not yet established. The skins potential as inexpensive sources of bioactive compounds, along with the growth interest for functional ingredients and with the current environmental situation that demands the reutilisation of wastes and by-products, justify its interest.

In order to characterise this by-product, pine nut skin was milled and separated with sieves of 0.5 mm (P50), 0.3 mm (P30) and 0.09 mm (P09). Fourier transform infrared (FTIR) spectra of the three particle sizes allowed to observe the similarity between the two smaller particle sizes, and the distinct profile of P50 throughout the 1800-600 cm^{-1} region. In order to relate this spectroscopic characteristic with the composition of the pine nut skin fractions, the three samples were Soxhlet-extracted with hexane, yielding 9.1, 6.6, and 7.9% (*w/w* in dry weight), from the higher to the lower particle size, respectively. The lipophilic extractives were characterised after derivatisation in trimethylsilyl esters/ethers, by gas-chromatography mass-spectrometry analyses. The lipophilic components identified and quantified were grouped in five major families: fatty acids, sterols, terpenic compounds, long-chain aliphatic alcohols, and secondary/secondary alkanediols. The presence of secondary/secondary alkanediols, namely 5,10-heptacosanediol, 4,10-nonacosanediol, 5,10-nonacosanediol, 6,10-nonacosanediol, 7,10-nonacosanediol, and 10,13-nonacosandiol, was 4 to 5 times higher in P50, totalling 229.3 mg/g of extract when compared with the remaining (83.8 and 51.6 mg/g for P30 and P09). Thus, the mechanical milling and separation of pine nut skin allowed to obtain a fraction rich in waxes, which could confer hydrophobicity in biobased materials. The composition on the other identified compounds was comparable within the three samples. Linoleic and oleic acids were the major compounds in all samples and, combined, represented at least 30% of the total content of identified compounds. Besides, the saturated fatty acids from C6:0 to C30:0 were detected, as well as β-sitosterol (25 to 29.1 mg/g), campesterol (3.9 to 5.8 mg/g), stigmasterol (0.5 to 2.3 mg/g) and stigmastanol (0.8 to 1.1 mg/g). Resin acids represented the class of terpenic compounds identified in pine nut skin. Both pimarane- and abietane-type resin acids were identified, predominantly pimaric acid and dehydroabietic acid in respect to each group of tricyclic diterpenic compounds.

Pine nut skin showed promising lipophilic composition, with a high abundance of mono- and polyunsaturated fatty acids, as well as with the presence of sterols, especially β-sitosterol, which is recognized as a hypocholesterolemic agent, besides other beneficial functions.

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Molecular weight analysis of *M. merluccius* and *P. glauca* collagen by LS-GPC: effect of temperature/stirring time on solubility

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The new reform of the Common Fisheries Policy (CFP) and several EU marine approaches such as *Blue Growth* and *2020 EU strategy* are focused on the development of sustainable socioeconomic and environmental growth in the marine and maritime EU region. To achieve this goal the valorization and biotechnological transformation of raw marine materials (discards and by-products) for the isolation/production of molecules that could be used in a high diversity of applications including antioxidants, anti-inflammatory, antifouling, biomarkers, etc., might be a useful tool. *Merluccius merluccius* discards due to Minimum Landing Size restrictions imposed by the Landing Obligation included in the new CFP and *Prionace glauca* skin byproducts are susceptible to be valorized based on the high collagen content of connective tissues. Collagen, the main structural protein in connective tissue, has a particular heterotrimeric structure which has been previously described for several species. As the structure of collagen and other proteins is determinant of its function, the final objective of this study is to analyze differences between *M.merluccius* and *P. glauca* collagen, to evaluate its potential use in cosmetics. Some differences on the collagen structure between teleost and chondrichthyes have been previously reported however there is a lack of detailed information regarding molecular weight (Mw) differences of α-chains determined by GPC-LS between these two groups. To study those differences the solubilization of collagen in the mobile phase of the chromatographic system is a key step. As the solubilization of collagen from *P. glauca* skin is more difficult than the one obtained from *M.merluccius* skin, the chromatographic behavior of both collagens under different temperature and stirring times was studied to test the influence of those factors on collagen solubility. Results showed that there is no influence of temperature or stirring effect on *M. merluccius* collagen solubility. Regarding *P. glauca* collagen, there is a positive effect of higher temperature on the solubilization of high Mw components in all the stirring times analyzed. Furthermore, no effect of stirring time increments is observed on the solubility of collagen if no temperature is applied. Besides, higher Mw components are observed when the sample is left stirring for 48 h and just after a temperature of 50ºC is applied for 15 minutes. Finally, higher molecular weight components are obtained after 48h of stirring time in the three temperatures/incubation times assayed.

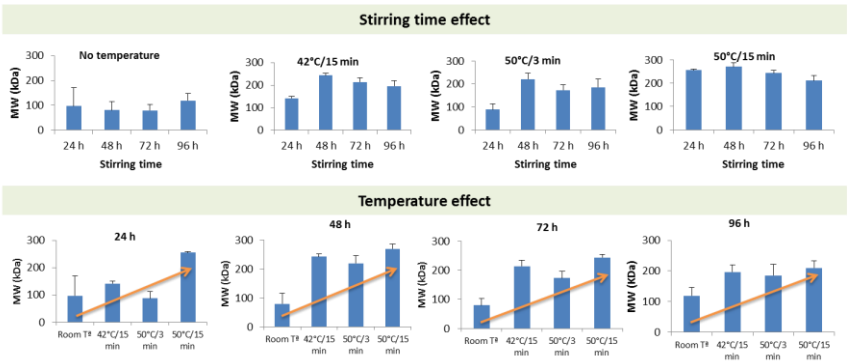

Figure 1. Effect of stirring time and temperature on molecular weight (Mw) of *P. glauca* collagen on GPC mobile phase under different temperature/stirring conditions.

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Aqueous extracts from *Agrocybe cylindracea* and *Pleurotus ostreatus* as source of antioxidant coatings

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Agrocybe cylindracea and *Pleurotus ostreatus* mushrooms are rich in polysaccharides and phenolic compounds with antioxidant and antimicrobial properties. Therefore, these mushrooms can be a good source of natural food preservatives and edible coatings. The main goal of this study was to develop an edible coating with antioxidant activity. Edible coatings are composed of biopolymers and may be carriers of additives with bioactive properties. This study developed an aqueous extraction process that allows the extraction of biopolymers and bioactive compounds (eg. phenolic compounds) from *A. cylindracea* and *P. ostreatus* mushrooms, with antioxidant activity, which can be used as preservative edible coating.

To accomplish this, after a pre-treatment by maceration of frozen mushrooms two consecutive aqueous extractions were applied, where the first was at room temperature (A) and the second (B) was hot extraction (90ºC; 1h; 5 000rpm). Extracts were lyophilized and the extraction yields were determined. Total phenolics content was determined through Folin Ciocalteu and antioxidant activity through ABTS method.

The yields of extracts A and B from *P. ostreatus* were 33.60% ± 0.39 and 15.18% ± 0.70. The yields of extracts A and B from *A. cylindracea* were 30.91% ± 0.89 and 14.77% ± 1.49, respectively. These results are in agreement with yields of *A. cylindracea* hot-water extracts reported by Tsai, Huang and Mau, 2006. Phenol content of extracts from *A. cylindracea* (extract A: 13.35 ± 0.55; extract B: 12.79 ± 0.67 mg gallic acid equivalent (GAE) per g of dry extract) were higher than phenol content of extracts from *P. ostreatus* (extract A: 10.28 ± 0.70; extract B: 11.52 ± 0.62 mg GAE per g of dry extract). The ABTS radical cation-scavenging activity was also higher in extracts from *A. cylindracea* (extract A: 12.04 ± 1.26; extract B: 6.78 ± 0.44mg ascorbic acid equivalent (AAE) per g of dry extract) than extracts from *P. ostreatus* (extract A: 6.39 ± 1.45; extract B: 5.75 ± 0.49mg AAE per g of dry extract). Results of this study indicate that *A. cylindracea* has more antioxidant activity than *P. ostreatus,* but both mushrooms are potential sources of natural antioxidant preservatives and polysaccharides to produce bioactive edible coatings.

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Potential of lemongrass and cardoon by-products as source of food bioactive compounds

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Lemongrass (*Cymbopogon citratus*) and cardoon (C*ynara cardunculus*) are agro-industrial by-products resultant from aromatic herbs and cheese industries, respectively. These industries generate lemongrass leaves that do not meet the required quality for infusions and cardoon's inflorescences, as only cardoon stigma top extremity is used for cheese production. The present work evaluates lemongrass and cardoon by-products regarding their carbohydrates and essential oil (EO) composition obtained by hydrodistillation. Phytochemical profiles of EOs were obtained by gas chromatography–mass spectrometry (GC–MS).

Lemongrass EOs yield was 0.66% which is in accordance with reported values from fresh leaves (0.73%) [1]. Twelve components were identified in lemongrass EO, being citral isomers the major components (neral 212.7 μg mL^{-1} and geranial 309.2 μg mL^{-1}), other oxygenated compounds were identified such as geranic acid 36.2 μg mL^{-1}, geraniol 31.6 μg mL^{-1}, and neric acid 7.1 μg mL^{-1}.

Concerning the cardoon, the flower was fractionated in stigmas, bracts and pappus in order to obtain the EOs, yielding 0.0534%, 0.00755%, and 0.00618%, respectively. Twenty seven components were identified, where palmitic acid was the major component of stigmas, bracts, and pappus: 342 μg mL^{-1}, 341 μg mL^{-1} and 320 μg mL^{-1}, respectively. Stigmas and bracts have also high amounts of linoleic acid (165.0 μg mL^{-1} and 125.3 μg mL^{-1}) and myristic acid (37.1 μg mL^{-1} and 23.9 μg mL^{-1}), respectively. However, pappus present different EO composition comprising also geranial (68.0 μg mL^{-1}), linoleic acid (56.6 μg mL^{-1}), and geranic acid (34.1 μg mg^{-1}). Simultaneously to the hydrodistillation process, an aqueous extract was obtained and recovered, yielding 15, 19, 8, and 5% for lemongrass, stigmas, bracts and pappus by-products, respectively, showing that lemongrass and stigmas aqueous extract displays a potential source of bioactive molecules, industrially discarded as a waste.

The high molecular weight material (HMWM) were obtained from dialysis (cut-off 12-14 kDa) of the aqueous extract, yielding 1.4, 0.9, 5, and 0.7% for lemongrass, stigmas, bracts and pappus, respectively, which makes bracts the most significant source of polysaccharides. The HMWM from lemongrass was composed of 21% total sugars, namely glucose (45%), arabinose (27%), and galactose (12%). Cardoon stigmas HMWM were composed of 30% total sugars, the polysaccharides were constituted by uronic acids (27%), glucose (22%), and arabinose (18%) whereas bracts HMWM contained 25% total sugars, namely uronic acids (32%), arabinose (26%), and glucose (22%) being the most significant source of pectic polysaccharides. Pappus presented 27% of total sugars, namely arabinose (40%), glucose (28%), and uronic acids (11%). Bracts water extracts were also composed of 2.6% free sugars and inositols, namely fructose (35%), myo-inositol (26%), arabinose (20%), and glucose (16%). In conclusion, lemongrass agro-industrial by-product EO holds citral content similar to fresh lemongrass EO suggesting a reliable source of citral (1.77g citral/ kg lemongrass by-product), with potential antibacterial activity. Lemongrass and cardoon by-products are a valuable bioactive compounds, such as terpenic compounds and pectic polysaccharides.

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Eggshells-derived calcium carbonate as lightweight filler for plastic formulations

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In last years, environmental rules for agrofood waste disposal have become stricter, making recyclability and reusability important issues for this industrial sector. On the other hand, to improve the plastics mechanical performance and concomitantly decrease their production cost, fillers as calcium carbonate are added to plastic formulations. However, its inherent high density limits the development of lightweight plastics. Eggshells are porous solid by-products produced by egg processing industry that contain 94% of calcium carbonate [1]. In this work, eggshells, supplied by Derovo SA, Pombal, Portugal, were used as raw material for the development of lightweight calcium carbonate due to its porous structure. Eggshells with and without organic membrane were milled using a ball milling processing and physicochemical and morphological properties of obtained powders was studied. For comparison, commercial calcium carbonate was also analysed.

Both eggshell powders, with or without membrane, showed FTIR profiles typical of calcium carbonate. X-ray diffraction patterns revealed the presence of crystalline calcite. The specific superficial areas (BET) were higher (6.5 m^2/g) than those observed for the commercial calcium carbonate (3.8 m^2/g) The particle sizes (3.4 μm) of the recovered powders were similar to commercial one (3.8 μm). The density was 2.38 g/cm^3, representing a decrease in *ca.* of 6% when compared with commercial calcium carbonate (2.54 g/cm^3). To further decrease the density, potato starch, recovered from potato by-products, was added to the formulations at the eggshells ball milling stage [2]. The presence of starch was confirmed by the FTIR characteristics bands in the range of 3680-3030 cm^{-1} and at 1008 cm^{-1}; and it did not change the calcium carbonate X-ray diffraction pattern. Despite the not so different specific surface areas (4.4 m^2/g) and particle sizes (6.0 μm) when compared to the commercial calcium carbonate, the density decreased in 8 and 16%, depending on the presence or not of the organic membrane, respectively.

Eggshells reveal to be an alternative and sustainable source of lightweight calcium carbonate filler for the plastics industry. Moreover, adding starch during the eggshells milling contributes to decrease the calcium carbonate density, offering a new strategy to develop lightweight fillers while adding value to agrofood by-products.

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Vitamin E in *Opuntia ficus-indica* (L.) Mill.: a comparative study between pulp and peel

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Vitamin E is a lipid soluble vitamin, designating a group of eight naturally occurring and structurally related vitamers, namely α-, β-, δ-, and γ-tocopherol and α-, β-, δ-, and γ-tocotrienol. α- and γ-tocopherols are the two major forms of this vitamin. The main dietary sources are vegetable oils, nuts and oilseeds. According to Regulation (EU) n.º 1169/2011, the dietary reference intake for adults is 12 mg/day.

The aim of this work was to evaluate the vitamin E content in the edible portion (pulp) and by-product (peel) of *Opuntia ficus-indica* (L.) Mill., which are commonly known as prickly pears.

Samples of prickly pears were obtained at Herdade de Peliteiros Silveiras, Montemor-o-Novo, Évora. After the separation between edible and non-edible portion, samples were homogenised and lyophilised. The chromatographic analysis of vitamin E was performed by high performance liquid chromatography. The chromatographic separation of the vitamers was achieved with a normal phase Supelcosil™ LC-SI (75 x 3.0 mm, 3 µm i.d.). A mixture of *n*-hexane and 1,4-dioxane (98.5:1.5) was used as eluent, at 0.7 mL/min. The detection was performed by diode array detection connected in series with the fluorescence detector (λexc: 290 nm; λem: 330 nm). The compounds were identified by comparison with standards and by their individual UV spectrum.

The most abundant vitamer in both parts of the fruit was α-tocopherol, being 47.97 and 208.9 µg/100 g of dry weight for pulp and peel, respectively. Nevertheless, the second major compound was β-tocopherol in the peel (9.32 ± 0.64 µg/100 g of dry weight) and γ-tocopherol in the pulp (9.35 ± 0.64 µg/100 g of dry weight). Concerning tocotrienols, only α-tocotrienol was possible to be quantified, since the other vitamers were not detected.

Based on the results obtained for the analysed prickly pear, it is possible to conclude that it is a good source of vitamin E and it could contribute to the intake of natural antioxidants. It is a fruit with nutritional interest and its use in human diet can bring potential health benefits to consumers. Also, due to the high amounts of vitamin E in the peel, these by-products could be added as natural extracts in cosmetic, pharmaceutical and food processing industries. Furthermore, this work will contribute to maintain the biodiversity and to promote the sustainable development and exploitation of these exotic fruits.

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Enhancement of lipase production by *Yarrowia divulgata*

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Lipase is a highly valuable compound which has a wide range of usability, for example in the food-, pharmaceutical- and beauty industries. *Yarrowia lipolytica* is known from its remarkably high lipolytic and proteolytic activity. In the last years some novel species belonging to the *Yarrowia* genus were described, and some of them also have the ability to produce lipase and other valuable compounds. The main aims of this research were to enhance the lipase production of some novel *Yarrowia* strains by using olive oil and Tween 80 supplements, and optimizing concentrations of olive oil.

Thirty-five strains of *Yarrowia divulgata, Y. porcina* and *Y. bubula* isolated from raw, grounded pork or beef were screened for lipase production by streaking them on the surface of Gorodkowa medium supplemented with olive oil. Inoculum (5%, v/v) was transferred into 500 mL flasks, containing 200 mL of fermentation medium (2% glucose, 0.64% peptone, 1% yeast extract) and supplemented with 1%, 5%, 10%, 20% and 50% olive oil and 0.05% Tween 80. Experiments were carried out for 72 or 148 hours at 28°C in orbital shaker (160 rpm). Samples were centrifuged and supernatants were used for measurement of extracellular lipase activity; additionally, yeast cells were disrupted before the quantification of intracellular lipase. 25mM p-nitrophenyl-laurate was used as substrate and the reaction was performed at 37°C in phosphate buffer (pH 7.2) for 10 min. Lipase activity was assayed spectrophotometrically at 405 nm. One unit (U) of lipase activity was defined as the amount of enzyme that releases 1 µM of p-nitrophenol per minute (pH 7.2, 37°C).

Almost all examined *Yarrowia* strain showed lipolytic activity, but *Y. divulgata* 5257 and 2062 were selected for further experiments. In the cases of *Y. divulgata* strains, 4.03 U/l and 8.11 U/l of extracellular enzyme activities were attained after 8 h and 48 h, respectively. Olive oil and Tween 80 have been published (Corzo & Revah, 1999; Galvagno et al., 2011) to enhance lipase activity and in our experiments this positive effect has also been proved: maximal lipase activity rised to 6,59 U/l and 26.00 U/l at 48 and 64 hours in the prescence of 1% olive oil, and to 83.47 U/l and 203.61 U/l at 72 hours when both additives have been added. The effect of olive oil concentration was also dinvestigated. *Y. divulgata* 2062 showed the highest extra- and intracellular lipase activity in the prescence of 10% olive oil, although in case of *Y. divulgata* 5257, 5 and 50% olive oil supplementation was the most successful in case of both intra- and extracellular activity, which is controversial to previously published data (Pignède et al., 2000; Coca & Dustet, 2006). *Y. divulgata* 5257 showed hight intracellular lipase activity (924.44 U/l), and *Y. divulgata* 156.73 U/l without Tween 80.

Y. divulgata strains exhibited the best performance and the addition of proper concentration of olive oil and Tween 80 led to the increase of lipase production. Thus besides *Y. lipolytica* members of other species may have great industrial potential and are worth to study.

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Olive pomace paste: a promising ingredient for food and cosmetic applications

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Over the last years, there has been a great concern about developing strategies for the valorisation of the residues generated by agro-industry. Olive pomace (OP) is a by-product of the olive oil production. It has been receiving special attention because of its environmental impact. Nevertheless, OP can be a source of phenolic compounds and other antioxidants [1] with potential as ingredient for food and cosmetics industries.

A patented OP processing [2] generates an olive pomace paste (OPP) as a secondary by-product. This study aimed to assess the OPP chemical profile in order to explore its potential for incorporation in new food and/or cosmetic products. A blend of four different OP (25% of each) - two from Trás-Os-Montes and two from Alentejo (Portugal) - constituted the sample in study. After mixing and applying a compression force (200-300 bar), a semi-paste was obtained and submitted to centrifugation. After removing the supernatant, OPP was collected and analysed.

Total fat, total protein, ash, and total dietary fibre were assessed according to AOAC methods [3] and the available carbohydrates content was calculated by difference. The vitamin E profile was analysed by HPLC-DAD-FLD [1]. For the extraction of the OPP antioxidants, a water/ethanol solution (20/80; v/v) and a stirring process was used (1h, 40 ºC). Total phenolics (TP) and flavonoids (TF) contents, as well as the antioxidant activity (by ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging ability) were analysed [4].

In dry weight, OPP presented a high content of total dietary fibre (37.5 %), available carbohydrates (36.9 %) and total fat (10.9 %). Total protein and ash amounts were 9.6 and 5.2 %, respectively. Considering the vitamin E profile, three vitamers were identified (α-tocopherol, β-tocopherol, and γ-tocopherol), being α-tocopherol the major one. The total content of this vitamin was 7 mg/100 g. Regarding the antioxidant activity, the FRAP and $DPPH^{\bullet}$ inhibition values were 67.9 mg ferrous sulfate eq./g and 49.5 mg trolox eq./g, respectively, probably influenced by TP and TF contents (35.9 mg gallic acid eq./g and 31.8 mg epicatechin equivalents/g, by this order).

Taking into account these results, the application of this secondary by-product as a functional ingredient in the food and cosmetic industries seems to have great interest, adding economic value to the olive oil industry. Moreover, the application of OPP allow the complete recovery of the OP potential. Thus, it accomplishes the “zero wastes” strategy associated with the circular economy concepts.

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Microwave assisted extraction of pine bark phenolics (Pinus pinaster Aiton subsp. atlantica): effect of solvent on extraction yield, total phenolic content and antioxidant activity

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Recently, special attention has been given to the addition of bioactive compounds to food products due to their antioxidant and antimicrobial activities. In addition to these properties, the bioactive compounds shown a wide range of physiological properties, such as antiviral, anti-carcinogenic, anti-inflammatory and in the prevention of cardiovascular diseases [1, 2]. Forestry industry by-products, such as pine bark, are sources of phenolic compounds, with known antioxidant and antimicrobial activities, having a great potential for application in foods [1, 3-5]. The objective of this study was to extract phenolic compounds, with antioxidant properties, from pine bark (*Pinus pinaster* Aiton subsp. *atlantica*) by microwave assisted extraction. An ETHOS X (Milestone, Italy) equipment was used (operating conditions:1600 W, 110 ºC for 30 minutes). The effect of the type of solvent on the yield of the process and on the antioxidant activity of pine bark extracts was also studied. The pine bark was dried to reach equilibrium humidity at 40 °C for 72 hours and milled to a particle's size of 200-850 μm. Three solvents were tested: water, ethanol and 50% water + 50% ethanol. The extraction yield was determined as the amount of solid extract recovered in mass compared to the initial amount of dry bark. Total phenolic content was determined by the colourimetric Folin-Ciocalteu method at 725 nm. The antioxidant activity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging methods. The type of solvent significantly influenced the extraction yield, the hydroethanolic mixture extracting a higher content of phenolic compounds (20.38±0.65% w/w), followed by ethanol (18.26±0.42% w/w) and water (12.81±0.13% w/w). No significant differences were observed in the total phenolic content, that were 72.03±1.49, 71.11±3.44 and 65.24±5.73 mg GAE/g sample respectively, with ethanol, 50% water + 50% ethanol and water. Significant differences were found in the antioxidant activity, by DPPH method, the hydroethanolic extract presented the highest value (781.46±40.74 μmol TE/g sample), followed by the ethanolic extract (588.70±5.91 μmol TE/g sample) and the aqueous extract (331.44±2.61 μmol TE/g sample). Regarding the ABTS method, no significant differences were found, and 497.32±54.39, 428.26±72.81 and 404.43±40.61 μmol TE/g sample were found in ethanolic, hydroethanolic and aqueous extracts, respectively. Overall, the best extraction solvent was the mixture 50% water + 50% ethanol.

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Free and total amino acid profile of coffee silverskin

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The global coffee production reached 169 million of 60 kg bags in 2108 [1]. Along with this production, a huge amount of residues is generated. They represent a source of contamination and environmental concerns, mainly due to their richness in phytotoxic and/or anti-nutrient compounds (*e.g.* caffeine, tannins, polyphenols...) that limits their direct use in soil or feed applications. However, they are a good source of bioactive compounds that can be extracted and used for several purposes. Therefore, the management of these high added-value residues can contribute not only to the sustainable development of the coffee chain, but also to the global economy and to a cleaner environment [2].

In this context, coffee silverskin (the main by-product of coffee roasting) emerges as a particularly interesting product. Previously, its richness in dietary fiber (~60%), caffeine and antioxidants was shown. It also contains a relevant amount of potassium, magnesium, and calcium [2]. To date, the amino acid composition of coffee silverskin was not known. The aim of this work was to study the free and total amino acid profiles of this by-product.

Coffee silverskin was gently supplied by a national coffee industry (BICAFÉ). Free amino acids were extracted with deionized water. To quantify total amino acids, acid hydrolysis was performed using 6 M HCl, at 110 °C for 24 h, after removing oxygen with a N_2 stream. For tryptophan quantification, an alkaline hydrolysis was carried out using 4 M KOH (110 °C for 4 h). A fast automatic pre-column derivatization (Jasco AS-4150, Japan) was performed combining *o*-phthalaldehyde/ 3-mercaptopropionic acid and 9-fluorenylmethoxycarbonyl chloride, in the presence of borate buffer (pH 10.2), using norvaline as internal standard. The derivatized amino acids were immediately analysed by reserved-phase HPLC.

The results show that arginine is the main free amino acid (138 µg/g), followed by glutamic acid (88 µg/g), aspartic acid (84 µg/g), and asparagine (69 µg/g). Free tryptophan was not detected in the sample and the sum of all free amino acids was about 600 µg/g. Regarding the profile of total amino acids, aspartic and glutamic acids were the most representative ones (14 and 12 mg/g, respectively). Silverskin also showed interesting amounts of proline (7.3 mg/g), alanine (7.7 mg/g), and branched-chain amino acids (BCAA) - leucine: 8.9 mg/g; isoleucine: 5.3 mg/g; valine: 6.4 mg/g. After hydrolysis, tryptophan levels reached 0.5 mg/g. The sum of total amino acids was about 109 mg/g.

In conclusion, besides dietary fiber, minerals, caffeine and antioxidants, silverskin is also source of relevant amino acids with potential to be used in food supplements. For instance, proline is important to the collagen structure and the BCAA contribute to muscle growth. Glutamic acid, tryptophan, and also proline contribute all to the neurological function [3]. The richness in these amino acids could lead to new valorization approaches of this by-product.

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The antinutrient γ-glutamyl-S-ethenyl cysteine in *Vicia narbonensis: influence of* accessions and growth stages

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The increase in world population, leading to an increase in food production, associated with climate change may result in a possible food shortage and risk in the availability of resources such as water and fertile soil. It is necessary to implement more sustainable, alternative and resilient agricultural practices for the global food supply [1]. Legume crops are associated with sustainable agricultural systems, such as crop rotations. Due to their ability to fix nitrogen in the soil, legumes contribute to soil fertility. *Vicia narbonensis* is a legume that grows very well in extreme climates with high yields and low cost. Due to its high protein content in the grain and straw (300 and 100 g/kg, respectively) it becomes a possible alternative for animal and human feeding. However, the presence of an antinutritional compound – γ-glutamyl-S-ethenyl cysteine (GEC) – in the *V. narbonensis* seeds can limit its consumption. This antinutrient acts as storage of nutrients for the plant and acts as a defense mechanism against predators, insects, among others, by giving a bitter taste resulting in a low palatability [2].

The objective of this work was to evaluate the GEC amount in different parts of the plant in order to know if the compound content changes with the plant growth: i) leaves, ii) pods (before the grain maturation) and iii) grains, in different accessions of *V. narbonensis*. GEC was analysed by reversed phase HPLC-DAD and the chromatographic peak monitored at 215 nm. As a commercial GEC standard is not available, seeds of *V. narbonensis* with high levels of GEC were used as positive control and *V. faba* as negative control. Therefore, the results will be expressed as relative percentage.

According to the results, the plants maturation lead to an increase on the antinutritional content: grain had higher GEC contents (44-194%), followed by the pods (0.8-84%). The lower content was found in leaves (0-8%). Although this trend was observed in all accesses, some of them presented much higher levels. Three accessions were randomly selected to verify if the growth of the plant influences the amount of GEC: two harvests were made for pods, with an interval of 3 weeks. It was also possible to verify an increase in GEC content with levels ranging between 9-79%, 21-69%, 28-59%, respectively. In the grain, it was only possible to make a single harvest, and the relative percentages of GEC in these accessions ranged from 129 to 181%. An increase in the GEC content from the pods to grain maturation was observed. In what concerns to the leaves (harvested every three weeks), different behaviors were detected according to the accession (GEC content increased or decreased).

For future work, there is a need to synthesize the pure γ-glutamyl-S-ethenyl cysteine as standard compound and, among the selected accessions with the lower GEC contents, to evaluate the possible natural selection in order to eliminate or reduce the antinutritional content at a range suitable for food.

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Antimicrobial activity of a functional ingredient from olive pomace

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Olive oil consumption and processing is increasing in Portugal and worldwide. Along with its production, several by-products are generated such as olive mill wastewaters and olive pomace (OP). The type of by-product produced depends on the method applied to obtain olive oil: three or two-phase system. In the three-phase system, water is added to the process. In the end, olive mill wastewaters are obtained, whereas in the two-phase system, no water is used. OP from the two-phase system is a semi-solid paste, mainly composed by fragments of olive skin, pulp and stone (solid phase), water and oil (liquid phase) [1]. It is known that OP is a rich and low-cost source of natural compounds with high interest for food industry. Therefore, several studies reported OP applications in food products [2]. Hence, the study of the OP antimicrobial activity is noteworthy having in view its use in innovative food products as a natural preservative, attaining the current consumers demand for natural additives.

This work aimed to assess the antimicrobial activity of an OP functional ingredient (OFI) obtained by a patented process [3]. Extracted physically from OP, OFI is a natural ingredient obtained without any chemical. Four samples from two-phase olive mills (2017/2018 season) were studied: two from the North of Portugal (Alfândega-da-Fé and Valpaços) and two from the South (Beja and Ferreira do Alentejo). The separation of the liquid phase (water and oil) from solid phase (olive skin, pulp, stone and kernel) was performed by application of a pressing force (200 - 300 bar). The obtained semi-paste was centrifuged and the supernatant (OFI) removed and lyophilized.

OFI samples at 500 mg/mL were screened in different agar methods (incorporation, surface spreading and disk diffusion) for the antimicrobial activity against *S. aureus* ATCC® 25923, *E. coli* ATCC® 25922, and *C. albicans* ATCC® 10231. For the evaluation of antimicrobial activity, cell suspensions (SC) were prepared in 1 mL of sterile 0.85% saline solution, with turbidity equivalent to 0.5 of the Mac Farland scale. A growth inhibition diameter greater than the one containing only solvent was considered as a positive result. Positive results were submitted to broth microdilution method in order to determine the minimum inhibitory concentration (MIC) [4].

All the extracts showed antimicrobial activity against *S. aureus* and *E. coli* strains, in one or more of the three screening methods used, with diameter zone inhibition between 7 and 15 mm for *S. aureus* and 9 to 12 mm for *E. coli*. No antimicrobial activity against *C. albicans* was verified with this methodology. MIC was determined for the four extracts for *S. aureus* and *E. coli*, with the best results for the extract from Alfândega-da-Fé (with 31.25 mg/mL and 62.5 mg/mL, respectively), followed by the extract from Beja (31.25 mg/mL and 125 mg/mL to *S. aureus* and *E. coli*, respectively).

According to these results, OFI showed antimicrobial activity against *S. aureus* and *E. coli*. This result corroborates the importance of OP as a source of value-added biologically active compounds for food and medical purposes.

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Study of elderberry juice industrial by-products for carbohydrates valorization

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Elderberry fruit is rich in sugars (18%) and anthocyanins (1%) [1] [2]. It is predominately used for juices production [3]. The processing generates by-products that can be valorized as a good source of valuable bioactive compounds and, simultaneously, minimizing environmental problems [4] [5]. The skins and seeds resultant from the pressing (pomace), as well as the compounds resultant from the clarification of the juice through the ultrafiltration process (retentate) are the most abundant by-products. Although the pomace as been proposed as a source of anthocyanins [4] [6], the retentate was never exploited neither the fibers and sugars that can be obtained from the pomace.

In the present work elderberry pomace and retentate were studied concerning their sugars content. Pomace was milled and freeze-dried before analysis, while the retentate, as a liquid slurry, was centrifuged to separate the water soluble from the insoluble compounds. The dialysis of the retentate water soluble material was performed to separate the polymeric compounds from the free sugars. Neutral and free sugars of elderberry pomace and the retentate fractions were analysed by GC-FID.

Elderberry pomace contains 50% of total sugars, in which glucose (50%) and xylose (24%) are the major contributors and are probably coming from the main anthocyanins present in elderberry skin (cyanidin-3-glucoside and cyanidin-3-sambubioside). Glucose can also be derived from cellulose and xyloglucans of the cell walls. Uronic acids represents 14% of total sugars and can arise from pectic polysaccharides. The retentate is also very rich in carbohydrates (46%) from which 23% are free sugars, mainly fructose (18%). Mannitol, a polyol, accounted for 8%.

The high molecular weight material accounts for 14% of the retentate water soluble compounds. The polymeric material consists of 53% sugars, mainly galacturonic acid (47%), followed by galactose (13%) and arabinose (12%), consistent with the presence of pectic polysaccharides branched by chains of galactose and arabinose. The presence of xylose (10%) and rhamnose (6%) is associated with polymerized anthocyanins namely cyanidin-3-sambubioside and cyanidin-3-rutinoside.

In the retentate, there are 13% of insoluble polymeric material, from which 50% is glucose, probably arising from the glycosyl moiety of the elderberry polymerized cyanins.

The data obtained shows that the retentate is a rich source of free sugars, namely fructose, and water soluble pectic polysaccharides, together with anthocyanins, that can be used as a coloured and sweet food ingredient. The elderberry pomace is a source of cellulosic fibers that, together with the presence of anthocyanins, can also be used as a coloured insoluble food ingredient.

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