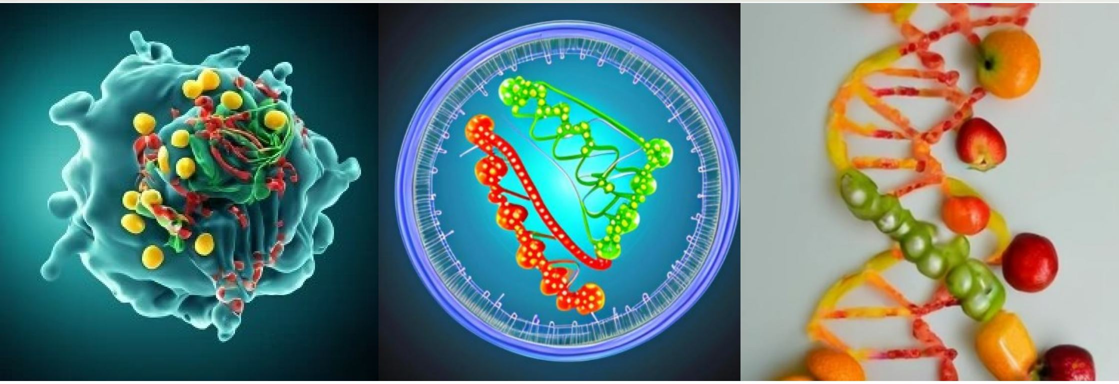
FoodOmics

2024

7th International Conference



2009-2024, fifteen years on from.
Where are we now, what's next.

14-16 February, Cesena (Italy)

FoodOmics 2024

7TH INTERNATIONAL CONFERENCE ON FOODOMICS

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14-16 FEBRUARY 2024

CESENA, ITALY

ABSTRACT BOOK

7TH INTERNATIONAL CONFERENCE ON FOODOMICS

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Department of Agricultural and Food Sciences
Alma Mater Studiorum Università di Bologna

PREFACE

The International Conference on FoodOmics was born fifteen years ago in Cesena as the first biennial forum on food, nutrition and health in which the most recent advances in scientific research are presented and discussed, based on a holistic approach ("omics") applied to food. In past editions, excellent speakers of clear international fame focused attention on hot topics and triggered a heated debate among public opinion and the scientific community.
The focus of each Foodomics edition is highlighted by the subtitle:

2009 – A science for nutrition, health and well-being in the post-genomic era.
2011 – The scientific forum where research and innovation meet.
2013 – FoodOmics, the science to discover.
2015 – Food to life.
2018 – From data to knowledge.
2020 – From Knowledge to Industry, from Industry to Knowledge.

The subtitle of this edition "2024 – Fifteen years later. Where are we now, what awaits us?" is therefore not accidental. In the past years we have acquired new knowledge, but are we sure we are interpreting it in the right way? Are we on the right path to arrive at a food system transition that will take us to the desired goals? This is the fil rouge that links the 4 sessions of this edition of FoodOmics, whose titles are not by chance always a question.
What's wrong and right with ultra-processed foods?
The onset of some diet-related pathologies is often associated with the consumption of industrial foods that have undergone extensive processing. Although epidemiological correlations between the consumption of ultra-processed foods and some diseases have often been reported, the cause-effect relationship is not yet convincing. FoodOmics holistic approach, which aims at a complete characterization of foods by coupling composition and structure, could help to correctly classify foods and interpret the results of

nutritional studies. The keynote speaker for this section will be Professor Ruud van der Sman (Wageningen University, NL), who will focus on the soft matter approach to food reformulation problems, explaining how the food industry faces the growing demand for reformulated products with high nutritional value. The soft matter approach offers the opportunity to decouple food microstructure from nutritional requirements, allowing foods to be optimized for health and sustainability without compromising palatability.

Food and health. Are we working from the right perspective?

The identification of the relationships between food components and clinical parameters that define the state of health is the future frontier, at the service of a politically correct definition of food safety, i.e. a correct and balanced diet accessible to all. The FoodOmics approach could help to give the most precise and accurate answers, also considering the great potential of artificial intelligence in discovering very hidden relationships between food consumption and health. The keynote speaker for this section will be Professor Elliot Berry (Hebrew University of Jerusalem, Israel), who will outline the multidisciplinary scientific approach to food policy and responsibility needed in disease prevention, as well as in individual, social and institutional resilience. In fact, only the prospect of a less conflictual and more harmonious world can lead to success in the fight against world hunger and food insecurity.

Are alternative sources of nutrients a reliable alternative?

The existence of the “food gap”, i.e., the increase of the amount of food that the world will require in 2050 if consumption were to continue unchanged, is well recognized and one of the main challenges linking humans and the environment is ensuring sufficient, nutritious, safe, and affordable food for all. One proposed solution is to replace conventional nutrient sources with other alternatives. The keynote speaker of this section, Professor Francesco Visioli (University of Padua, IT) will focus on a critical analysis of alternative sources of nutrients that lie on the ridge between sustainability, emotion, and science. In fact, although a diet rich in alternative plant foods has long been associated with improved health, when it comes to calculating sustainability things are not as simple as the specialized press often reports. Alternative sources cannot become a viable alternative without knowing more. FoodOmics methodological approach certainly has what it takes to shed light on this enigma.

Nutrient bioaccessibility and bioavailability. Do we need a new nutrition label?

By reading the “nutritional facts” label, the consumer is convinced that everything contained in a food product is absorbed in the intestine, without any interference from the matrix during digestion. On the contrary, the structural dimension of a food, very complicated and rarely measured, affects the accessibility and bioavailability of nutrients. The FoodOmics approach based on in vitro model systems goes beyond the chemical composition of foods and considers bioaccessible and bioavailable components, which represent the most effective fraction of food. During the fourth and final session, keynote speaker Professor John Van Camp (University of Ghent, BE) will focus on designing advanced cell culture models to study the bioavailability and bioactivity of food-derived components. The hope is that in the not-too-distant future the nutrition facts label will consider bioaccessibility and bioavailability of the nutrients present in a specific product.

We are certain that the keynote speakers, the speakers selected for the oral presentations and the poster presenters will be able to make this edition of FoodOmics a stimulating event that goes beyond the state of the art. Thank you all for your participation and keep in mind that nothing would have happened without the help of the Scientific Committee and the Organizing Committee. In this regard, we would like to sincerely thank our colleague **Elena Babini**, Chair of the Organizing Committee.

Alessandra Bordoni **Francesco Capozzi**

co-chairs of the Scientific Committee

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FoodOmics 2024

KEYNOTE SPEAKERS ABSTRACT

Soft matter approach to food reformulation issues.

Ruud G. M. van der Sman

Food & Biobased Research, Wageningen University & Research, The Netherlands

Currently food industry is facing big challenges as consumer concerns about sustainability and health are calling food reformulations towards lower amounts of sugars, sodium, fat, animal protein, but higher amounts of food fibers, and plant-based ingredients. Many of the foods are developed in a traditional, artisanal way, without a clear insight about ingredient functionality, and why processing is performed in a certain way. Hence, current food reformulations practices are still largely a matter of trial-and-error.

Via the application of theories from soft matter physics to food materials in the last decade, we have learned important lessons for approaching food reformulation issues. These lessons are primarily derived from the premise that food microstructure/texture can be realized without much dependency on precise molecular details, but depending on coarse-grained molecular properties. Hence, we pose that similar food microstructures can be realized with multiple choices of food ingredients. As a central hypothesis for food reformulation, we pose that similar texture or original foods can be realized with reformulated foods if one can exactly mimic the food rheology and phase transitions. For structuring of foods phase transitions like starch gelatinization, protein denaturation, boiling or freezing of water are important. Via application of soft matter physics we have learned which physical coarse-grained properties drive the food rheology and phase transitions.

We will give a detailed illustration of our soft matter approach with two examples: sodium reduction in expanded starchy snacks, and sugar reduction in sweet bakery products. Their processing will be illustrated with the processing path in the state diagram, showing all relevant phase transitions during the food processing. Surprisingly, both phase transitions and rheology are governed by similar coarse-grained molecular parameters, such as the volumetric density of hydrogen bonds. For the sugar reduction issue we show that multiple ingredient solutions can render similar texture as the original foods. Moreover, we show that these ingredients can even be sourced from unutilized side-streams of the agribusiness, rich in cell wall materials.

We finalize with presenting results in formulation of novel foods like plant-based meat analogs and 3D printed foods, where the foods have to be formulated from scratch. We show that the rheology of several classes of food materials, but having a multitude of ingredients, can be mapped to a select number of master curves, showing validity of the premise of soft matter physics that same texture/rheology can be realized by a multitude of ingredients. Moreover, the rheology is driven by same coarse-grained parameters which govern our sugar reduction strategy. Thus the soft matter premise offers opportunity to decouple texture from nutritional details, leaving opportunity to optimize foods for health, sustainability and other consumer concerns.

Pandemics, Plenty and Poverty: the fight against Malnutrition and Food Insecurity.

Elliot Berry

Former Director Braun School of Public Health, Hebrew University-Hadassah Medical School, Jerusalem, Israel

The current world geo-political situation, following so soon after the COVID pandemic, has led to major food, energy, water and humanitarian crises for which the world is poorly prepared. The current tragedies in Ukraine and the Middle East show how food and energy may be used as political weapons and instruments of war. Poverty and War may be considered as epidemics with long-term consequences, prolonged, in part, by human choice and bystander indifference. All this is occurring in a world where one-third of food is wasted, yet there is enough food to feed everyone. Approximately, equal numbers suffer from Under-nutrition and Over-nutrition, the latter a pandemic far greater than COVID. The basic causes of both are food Insecurity and poverty. Unequal distribution and economic interests are given higher priorities than equity and social justice.

However, this grave situation may be leveraged to produce positive responses at national and international levels to align food systems with the Sustainable Development goals. Operationally, a major re-thinking is required of the policies and science included in these multi-disciplinary activities. We discuss these challenges and suggest some new approaches using the sociotype framework.

In practical terms, we do need not more recommendations but actions on the following points: 1) **Responsibility**: Food Security is a fundamental human right and must be a basic Government responsibility (as for health, education, and defense). There must be access for all to affordable, safe and healthy food. 2) **Prevention** and **Preparedness**: Multi-disciplinary teams and international partnerships have to be established with defined leadership and dedicated budgets for the next pandemic / crisis – *which will surely come* 3) **Policies**: There must be long-term SYSTEMIC policies towards Complex Adaptive Sustainable Food Systems. 4) **Resilience**: Building resilience through the Sociotype Framework – at Individual, Relationships (Society) and Context (Institutional) Levels.

The goals are to eradicate world hunger and prevent disease, with the additional prospect of fewer conflicts and a more harmonious world - all crucial to combat malnutrition and food insecurity.

Nutrient sources and sustainability: beyond the hype.

Francesco Visioli

Department of Molecular Medicine, University of Padova, Italy

The agricultural and food industry has a strong impact on the environment, both in terms of water and land consumption and greenhouse gas emissions. With increasing prosperity, total calorie consumption in countries increases, with meat consumption in particular on the rise. It is therefore necessary to reduce food waste and find different ways to produce more food worldwide. One solution that has been proposed is to replace animal protein sources with plant-based ones. Certainly, a plant-based diet has long been associated with better health, but when it comes to calculating sustainability, things are not as simple as the lay press often reports.

This talk is about the tension between the need to reduce environmental impact and providing quality nutrients to a large and growing number of people emerging from poverty.

1. Animal- and plant-based protein sources: a scoping review of human health outcomes and environmental impact.. Ferrari L., Panaite S-A., Bertazzo A., Visioli F. Nutrients (2022) 14:5115. DOI: 10.3390/nu14235115

2. Plant-based diets reduce blood pressure. A systematic review of recent evidence. Tomé-Carneiro J., Visioli F. Current Hypertension Reports (2023) 25:127-150. DOI: 10.1007/s11906-023-01243-7

Design of advanced cell culture models to study bioavailability and bioactivity of food derived components.

John Van Camp

Department of Food Technology, Food Safety and Health, Faculty Bio-Science Engineering, Ghent University, Belgium

An overview will be given of methods and *in vitro* models used in our laboratory to evaluate the bioavailability and bioactivity of food-derived bioactive components. Human cell models based on monocultures, cocultures, barrier models and spheroids to study the gut liver axis, where applicable also combined with cell lines from other tissues (blood vessels, immune system, muscles) will be presented. Our major functional tests include measurement of bioavailability and bioactivity, with specific attention to bioenergetics, proliferation and toxicity assays. During the presentation, examples will be given from our work with bioactive peptides, plant metabolites (polyphenols), and rare sugars.

FoodOmics
2024

SELECTED ORAL COMMUNICATIONS
ABSTRACTS

Session 1: What is wrong and right with ultra-processed foods (UPFs).

Untargeted characterization of short peptides in complex food matrices.

Marie Yammine[1,2], Marc Haegelin[1], Fabrice Bray[1], Juliette Caron[2], Christian Rolando[1,3] and Isabelle Mouly[2]

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Food products are complex mixtures with a diverse macromolecular composition, such as polyphenols, carbohydrates, lipids, and proteins, as well as their hydrolysis-derived structural entities including phenols, oligosaccharides, fatty acids amino acids and short peptides composed of up to four amino acids. Short peptides are of significant interest due to their exceptional nutritional value, organoleptic properties, and health-promoting features and their identification and analysis in complex food matrices is crucial. However, compared to free amino-acids, the analysis of short peptides presents considerable challenges due to their small size and extreme physico-chemical features. Adequate separation by liquid chromatography and detection by mass spectrometry is difficult, and classic omics workflows do not typically capture them.

This work presents a workflow for identifying and quantifying short peptides in fermentation-derived food matrices using chemical labelling. The raw data was processed using an in-house Python program, enabling reliable identification of short peptide sequences based on MS/MS spectra, specific fragments and reporter ions mapping. The workflow allows the characterization of short peptides in various food matrices derived from fermentation-related hydrolysis. For instance, we successfully identified 500 short peptides in commercial yeast extract and detected chemical modifications, such as oxidation of methionine and cysteine. A patent application for this workflow is filed and submitted.

Combining the reactivity of gold nanoparticles and proteomics approaches to address protein-protein interactions in food proteins.

Davide Emide[1], Giovanni D'Auria[2] Chiara Nitride[2], Gianfranco Mamone[3], Pasquale Ferranti[2], Francesco Bonomi[1], Stefania Iametti[1] and Alberto Barbiroli[1]

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[3]ISA, CNR Avellino, Italy

The thiol-binding capacity of Au nanoparticles (AuNPs, 20 nm) was used to test accessibility of protein thiols in wheat flour, where proteins interact through both hydrophobic interactions and disulfide bonds. At difference with soluble thiol reagents, the AuNPs size limits their penetration in protein networks. Also, the stability of the thiol ligation to AuNPs simplifies removal of unbound proteins and recovery of AuNPs (and of covalently bound proteins) through centrifugation, making it possible to identify AuNPs-bound proteins through MS/MS and limited proteolysis. Low concentrations of detergents allowed controlled breakdown of hydrophobic interactions and allowed to distinguish binding of AuNPs to insoluble network-forming proteins rather than to soluble ones. Both glutenins (having free cysteines and intramolecular disulfides) and gliadins (no free thiols and only intramolecular disulfides) were found to be covalently bound to AuNPs, proving that the two protein classes form intermolecular disulfides already in the flour. These results pave the way to further studies on the time course and the molecular determinants of protein-protein interactions during deposition in the seed or on the role of some proteins or protein classes in gluten formation. Use of AuNPs of different size should also make it possible to assess the geometry and the structure of the protein network in foods, either wheat-based or not.

Supported in part by the National Recovery and Resilience Plan (NRRP, Call No. 341 of 15/03/2022) through the project: "Research and innovation network on food and nutrition: Sustainability, Safety and Security" (ON Foods).

The wholewheat pasta: expectation vs. reality.

Federica Pasini[1,2], Silvia Marzocchi[1], Marcello Greco Miani[3] and Maria Fiorenza Caboni[1,2]

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Grain-based foods have a relevant role in the human diet, providing much of the energy and nutrient needs, mainly proteins and carbohydrates. Many epidemiological studies cite the health benefits associated with the consumption of whole grain (WG) foods, since many components important for human health (dietary fiber, phenolics, folates and other phytochemicals) are located in the outer layers of the grain [1]. Nowadays, the definition of WG when used as an ingredient is quite well established and many international entities all agree that a WG includes all of the bran, germ, and endosperm of the original grain kernel in their original proportions [2], but there is far less clarity and consistency on what a WG food is. A wide range of opinions and government standards exists on when a food qualifies as "whole grain." It is important to define high and nutritionally significant minimum levels of WG ingredients for WG foods and provide clear front-of-pack information about the amount of WG in the product and on the partial replacement of WG with other ingredients such as refined wheat. Besides, in the multi flow process of the modern milling system, the various millstreams are separated for recombination at a later stage to become WG flour with a bran, germ, and endosperm proportion characteristic of the original grains. However, recombination per grain and per variety will result in some fluctuations in the ratios of endosperm, bran and germ between batches of flour and products.

For these reasons, the aim of this study was to show the quali-quantitative composition of the lipidic fraction of “pasta integrale” currently present on the Italian market. Differently from previous studies, commercial wholegrain pasta samples have been characterized for their lipid fraction, which is mainly concentrated in the germ of the kernel [3,4]. Data obtained showed a great variability in lipid composition and oxidative status of the WG pasta samples, suggesting a different WG flour amount and composition in the final products. Besides, the results support the need to provide markers for an assessment of the grain tissue ratios on recombined flour, in order to provide the consumer with clear information.

[1]. Barrett EM, Batterham MJ, Ray S, Beck EJ. Whole grain, bran and cereal fibre consumption and CVD: A systematic review. Br. J. Nutr. 2019; 121: 914–937.

[2]. van der Kamp JW, Poutanen K, Seal CJ, Richardson DP. The HEALTHGRAIN definition of ‘whole grain’. Food Nutr. Res. 2014;.58:22100.

[3] Marzocchi S, Messia MC, Marconi E, Caboni MF, Pasini F. Lipid process markers of durum wheat debranning fractions. Foods 2023; 12:3036.

[4] Marzocchi S, Caboni MF, Greco Miani M, Pasini F. Wheat Germ and Lipid Oxidation: An Open Issue. Foods 2022; 11:1032

Malting process on legume seeds: an NMR study to observe the effects on nutritional properties.

Mattia Spano[1,2], Giacomo Di Matteo[1,2], Alessio Cimini[3], Alessandro Poliziani[3], Anna Maria Giusti[4], Lorenzo Maria Donini[4], Lorenzo Morgante[3] and Luisa Mannina[1,2]

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Malting is a multistep process used to produce barley malt, mainly applies in brewing technology. However, basic aspects of malting can be applied to a wide range of plant seeds, such as legumes. Legume seeds are one of the world's most important sources of nutrients [1]. However, legume seeds such as chickpea, lentil, beans, peas, and soybeans are difficult to process since the long boiling times required, the resistance to mechanical damage, and the high levels of anti-nutritional factors namely phytic acid, tannins, oligosaccharides, enzyme inhibitors. Among the several nutritional and technological advantages of malting process, the reduction of anti-nutritional compounds in favor of nutritional ones is emerging as potentially effective [2].

In the present work, the effect of malting process on legume seeds produced in Lazio region was observed by both NMR-based untargeted and targeted approaches. Several metabolites belonging to different chemical classes were identified in both hydroalcoholic and organic Bligh-Dyer extracts. Moreover, ^{31}P NMR experiments were used to identify and quantify phytic acid. The observed reduction of anti-nutritional factors confirmed the efficiency of malting process in improving legume seed nutritional properties.

[1] A. Gasiński qt al. LWT 169, 2022
[2] G.Caprioli, et al. Food Chem. 192, 2016

Shimadzu's ready-to-use method for Metabolomics. Differentiation of Tomato Varieties.

Domingo Pastran

Shimadzu Italia S.r.l.

Metabolomics analysis can be a challenge in analytical laboratories, especially for newcomers to the field. Shimadzu Corporation offers an extensive array of instruments and application tools to simplify and enhance efficiency analysis, so users can begin a food metabolomic study without time-consuming evaluation of method parameters. Shimadzu's LC-MSTOF and LC/GC-MSMS systems together with the *Multi-Omics Analysis Package* supports the entire metabolomic analysis process: from sample preparation to result and data interpretation, including all chromatographic and MS instrumental settings. Different varieties of tomato were studied using Shimadzu's Package and a GC-MSMS system. Results clearly categorized four types of tomatoes and highlighted their differences in amino acids and sugars content, demonstrating this method package as a useful tool for the metabolomic analysis of foods. Moreover, untargeted analysis of metabolites can be performed using Shimadzu’s QTOF systems and, unlike other technologies such as ion trap instruments, it can be developed for quantitative analysis to establish and quantify biomarkers for each sample. All chemometrics tools: PCA, HCA, Volcano Plots and other multivariate statistical plots are automatically generated using Shimadzu's platform, as well as metabolic mapping, network analysis and projection on the metabolic map.

This eliminates the need for the operator to manually configure the whole analytical and data processing method.

Furthermore, visualization of quantitative changes in metabolites can be easily done through metabolic mapping, network analysis and projection on the metabolic map.

Application of FFC NMR in Food Science: Overview and Examples.

Anastasiia Nagmutdinova[1], Leonardo Brizi[2], Villiam Bortolotti[1], Gianni Ferrante[3], Germana Landi[4] and Fabiana Zama[4]

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During different processes that food products undergo structural matrices could change drastically, thus modifying such properties as quality, nutritious values, taste, health benefits, stability, etc. Furthermore, product authenticity could be explored by the studies of structure.

Considering that the structure of the food systems could be partially explained by the molecular dynamic of water, the Nuclear Magnetic Resonance (NMR) Relaxation technique is a useful tool in food science for the exploration of the correlation times of the nuclear dipolar fluctuation, which are responsible for NMR relaxation. Here we present the FFC (Fast Field Cycling) NMR technique, the first commercial instrument of which was created and placed on the market in the late 1990s by Stelar Srl (Mede, Italy). FFC is a new complimentary tool that through previous years has shown good results in food investigation. For example, with FFC it was possible to distinguish structural differences in cheese prepared with pasteurized and unpasteurized milk. FFC NMR, in contrast with NMR, measures T1 relaxation time at different Larmor frequencies, which allows the exploration of molecular dynamics, transport, diffusion of solvents, and chemical exchange.

FFC is a noninvasive and nondestructive technique and measurements could be performed as it is for solid or liquid samples without additional pre-treatment.

Here, we present a brief overview of the theory behind the FFC technique, experiment setup, data processing methods, and an overview of the previously conducted research. We show some experiments on different types of Mozzarellas and Pecorino cheese to demonstrate the potential of FFC NMR in food studies.

To process or not to process? The good, the bad and the ugly sides of processing complex carbohydrates for different consumers.

Maayan Ben-David, Gil Refael, Sivan Isaschar-Ovdat, Shlomit David, Hila Tarazi Riess, Carmit Shani Levi and Uri Lesmes

Dept. of Biotechnology and Food Engineering, Technion – Israel Institute of Technology, Israel

The global appetite for sustainable, diverse and high-quality foods can be met by science-driven control over their complex compositions, properties as well as intricate digestive fate. This talk will seek to highlight the role of scientific research in the rational design of healthier food choices. It will present the utility of *in vitro* digestion models to elucidate the complexities of digestive trajectories of complex carbohydrates, such as starch and dietary fibres. First, we will demonstrate the ability to process starch into nano-sized architectures for functional foods that control the delivery of bioactives, such as capsaicin and curcumin to the small intestine (1,2). Second, we present digestomics evidence on the differential anti-nutritional effects of carrageenan on digestive proteolysis of food beverages in infants, adults and seniors but NOT in the case of processed meats (3–5). These effects are governed by electrostatic biopolymer interactions, consumer age and the food matrix properties. Last, we will discuss novel food sources, such as Chitin from crickets that could offer new tools to shape the diversity of the human colon microbiota (6). Thus, this lecture will give attendees food for thought on the need for evidence-based rational design of foods that balance benefits and possible risks to meet the needs of different consumers.

Session 2: Food and health. Are we working from the right perspective?

New Frontiers in Metabolic Health: Mitochondrial Health Based Nutritional Ingredient for Cognitive and Muscle Health.

Larisa Andreeva

Mitocholine, Ltd

Mitocholine, Ltd is a science organization developing a nutritional fortification platform of ingredients to support physical wellbeing, particularly for healthy aging.

A vital function of every cell in our bodies is the process of metabolism. For our cells and bodies to function, we need access to an energy-carrying molecule called adenosine triphosphate (ATP). Sadly, as we age our body's ability to produce sufficient ATP from our daily diet diminishes and our physical and mental wellbeing declines in parallel. However, the mechanism of efficient ATP generation for glucose intense brain and muscle is scientifically sound, opening the possibility for effective food supplement to be developed that maintain and support mitochondrial ATP generation through cellular glucose metabolism. Mitocholine was founded on the discovery of synergistic action of mitochondrial substrates that, when taken orally, maintain intracellular ATP generation through effective use of glucose by counteracting insulin resistance.

Early stage research shows that our formulations could provide clear benefits to a person's physical energy level and exercise capacity. While these benefits are important for athletic performance, they are particularly important to maintain strength and mobility in the aging population. Also associated with aging is reduced ATP production in the brain which, in comparison to muscle aging, is further exacerbated by neuronal insulin resistance, creating a brain energy gap. What we have also discovered is that, due our ingredient's unique ability to increase the brain's metabolic receptiveness to insulin, it successfully brings more glucose into brain cells and, like muscle, also uses it more effectively. Thus, our first ingredient has been named MiBrain™.

This presentation will give an overview of MiBrain™ composition, mode of synergistic action, and preclinical and clinical supporting data.

[1]. CUNNANE ET AL, BRAIN ENERGY RESCUE, NATURE REVIEW, 2020
[2]. WWW.MITOCHOLINE.COM

Associations between sheep meat intake frequency and blood plasma levels of metabolites and lipoproteins in healthy Uzbek adults.

Diyora Kurmaeva[1], Yongxin Ye[2], Inal Bakhytkyzy[2], Violetta Aru[2], Dilbar Dalimova[2], Shahlo Turdikulova[2], Lars Ove Dragsted[2], Søren Balling Engelsen[2] and Bekzod Khakimov[2,*]

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Uzbekistan is one of the countries with the highest number of diet-related chronic diseases, which is believed to be associated with high animal fat intake. Sheep meat is high in fats (~5% in muscle), including saturated and monounsaturated fatty acids, and it contains nearly twice the higher amounts of n-3 polyunsaturated fatty acids and conjugated linoleic acids compared to beef. Nevertheless, sheep meat is considered health promoting by the locals in Uzbekistan and it accounts for around 1/3 of red meat intake in the country. The aim of this study was to apply a metabolomics approach to investigate if sheep meat intake frequency (SMIF) is associated with alterations in fasting blood plasma metabolites and lipoproteins in healthy Uzbek adults. The study included 263 subjects, 149 females and 114 males. For each subject a food intake questionnaire, including SMIF, was recorded and fasting blood plasma samples were collected for metabolomics. Blood plasma metabolites and lipoprotein concentrations were determined using 1H NMR spectroscopy. The results showed that SMIF was confounded by sex, body mass index (BMI), and age, in ascending order ($p < 0.01$). Multivariate and univariate data analyses showed differences in the levels of plasma metabolites and lipoproteins with respect to SMIF. The effect of SMIF after statistical adjustment by sex, BMI, and age decreased but remained significant. Pyruvic acid, phenylalanine, ornithine, and acetic acid remained significantly lower in the high SMIF group, whereas choline, asparagine, and dimethylglycine showed an increasing trend. Levels of cholesterol, apolipoprotein A1, as well as low- and high-density lipoprotein subfractions all displayed a decreasing trend with increased SMIF although the difference were not significant after FDR corrected.

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Metabolomics for the sustainability of a meat value chain: application to the Italian heavy pig production system.

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Italian Protected Designation of Origin (PDO) dry-cured hams produced in Italy constitute a value chain of more than 3 billion € per year. Heavy pigs, slaughtered at about 160 kg of live weight and at 9 months of age, are the animals that produce the raw materials (i.e. the legs) that are then processed to obtain these typical hams. Legs can be properly processed to become PDO hams only if the pigs are raised following some specification rules and derive from a breeding program that has meat and carcass quality traits as its main objectives. These traits are defined by a complex interplay of biological mechanisms and interactions, most of which are still to be defined. Different breeds and lines, and within lines, males (castrated) and females, are characterized by a wide range of variability among these production traits. To understand the basic biological processes underlying economic relevant traits that are routinely evaluated in the Italian heavy pig breeding program, we investigated targeted and untargeted metabolomic profiles of the animals. Plasma from a total of about 800 Italian Large White and Italian Duroc pigs was analysed with targeted and untargeted metabolomic platforms to obtain information from about 1000 metabolites. Data were then initially modelled via univariate and multivariate statistical approaches to identify metabolites characterizing and discriminating the two breeds and the two sexes. Then, metabolomic data were within-breed and sex analyzed via a systems biology approach to obtain biological networks that were further compared to identify breed and sex specific metabolite routes. The obtained results could help to shed new lights on how different genetic backgrounds may explain the basic biological factors that contribute to the variability of meat and carcass quality traits in heavy pigs.

From EAT-IT to MED_EAT-IT: a step towards sustainable personalized diets for the Italian food context.

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The EAT-Lancet Commission proposed a set of guidance (the Planetary Health Diet) that should be adapted to the different food contexts to develop country specific Sustainable Healthy Diets. A first attempt of adaptation to the Italian food context resulted in the EAT-IT dietary pattern we developed and assessed for its nutritional adequacy, feasibility, and environmental impact, underlying different criticalities. In this study we aimed at improving the dietary pattern to solve the nutritional and acceptability issues and increase alignment with the Mediterranean Diet (i.e. the MED_EAT-IT pattern). Also, strategies of adaptations of this pattern to different energy targets were considered demonstrating increased inadequacies when reducing the energy intake. A calculation tool was developed to enable diet optimization (with outputs in terms of nutritional profile, and Carbon and Water Footprints) towards the promotion of personalized nutrition. Strategies and tools like those proposed here could be helpful for the improvement of sustainable dietary plans and for the identification of potential issues that could prevent their adoption. The approach is now applied within the INSTEAD study devoted to the validation of the MED_EAT-IT pattern through a randomized controlled intervention in adult volunteers.

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Cultivar-dependent responses to different farming methods: a 1H NMR-based metabolomics study in table grapes (cv. Vittoria and cv. Timpson).

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Farming methods can significantly affect the metabolites in foods, as a plants response to different stimuli. This is a very actual issue, considering the renewed attention to food security and quality, as well as to agroecosystems health. Grapevine is one of the most cultivated crops in the world and table grapes are largely consumed foodstuffs, with appreciated nutritional and sensory features. In the present work we performed, by ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) combined with multivariate statistical analysis (MVA), a characterization of the metabolic profiles of table grapes from biodynamic (BD), organic (ORG) and integrated (INT) agriculture. Therefore, this study may represent a useful completion of the investigations already carried out on table [1] and wine [2] grapes. NMR analyses involved juice samples from grapes *cv. Vittoria* (cultivated with BD and INT treatment; vintage 2019) and *cv. Timpson* (produced with BD, ORG and INT agriculture; vintage 2020). In both study cases (*cv. Vittoria* and *cv. Timpson*), the preliminary unsupervised analyses revealed as the different farming methods constitute significant drivers for samples differentiation. In the supervised analyses, the most susceptible metabolites to the treatments, for both cultivars, appeared approximately the same in each of the performed pairwise comparisons of farming methods. Very similar discriminant metabolites resulted in our previous findings for *cv. Italia* grapes, where a specific comparison of BD, ORG and INT samples was carried out in a 3-year period experiment [3]. Fructose, α-/β- glucose, malate, citrate, arginine, glutamine, alanine and proline resulted the most viticultural practices-dependent metabolites. Nevertheless, the slightly different statistical trends observed, comparing the outcomes, also suggest specific cultivar-dependent responses to farming methods.

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Exploring wine characterization: variety and sensory properties.

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Nuclear magnetic resonance spectroscopy (NMR) is currently recognized as an important tool in food authentication and food fraud detection gaining importance alongside automated algorithms. We aimed to explore the application to assess the wine origin, variety and sensory properties. Two sets of spectra of Czech wines, accompanied by the information about producer, variety, origin, year of harvest, and chemical profile analyzed via OIV-approved methods [1], were analyzed. The first set of 2857 wines was used to create a statistical model for categorizing wine types and varieties, while the second set of 286 wines aimed to reveal correlations between sensory properties and taste profiles. Samples (540 µL) were mixed with 60 µL of phosphate buffer (1.5 M, pH 4.0, 0.2% NaN_3 and 5 mM DSS). Spectra were collected at 500 MHz spectrometer, at 298 K using *noesypr1d* pulse sequence, standard settings and processing. A random forest model achieved reasonable accuracy in classifying varieties, such as 96% for Pinot Noir, 96% Blaufränkisch and 92% for Riesling. Cluster analysis revealed clustering of genetically- and sensorially similar varieties. Correlation analysis revealed significant associations with sensory properties, such as tropical fruit taste was positively correlated with acetate, proline, and negatively with isopentanol. Acknowledgment: METROFOOD-CZ (MEYS Grant No: LM2023064; LM2018100).

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Session 3: Are alternative sources of nutrients a reliable alternative?

Natural bioactives for a more sustainable food and health care system.

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Natural bioactives present in edible plants and foods are part of whole diets, ingredients, or supplements, are vital for human and planetary health, and can positively influence multiple aspects of human health, wellbeing, and performance [1]. They are classified into micronutrients (vitamins, minerals), phytonutrients, bioactive peptides, and pre-, pro-, post- and synbiotics. These bioactives act in concert, interact with the human host and its (gut) microbiome, and exert their individually subtle, yet combined significant effects over extended periods of time [2].

The future of translational science on natural bioactives is currently being developed along the following lines: systems-level rather than reductionist approaches to understanding their functions and interactions; and the leverage of omics sciences, computational biology, artificial intelligence and smart screening for discovery and validation of these compounds [1].

All this is fertilizing Personalized and Precision Nutrition, advancing the confluence of nutrition and medicine, and is thereby supporting more efficient, yet affordable healthcare solutions that better use food for health maintenance and disease prevention [3,4].

Hence, researching and investing in natural bioactives contributes to improving and sustaining our health care systems, protecting biodiversity, while creating added and shared value for consumers, patients, and interest groups [1]. This transition is crucial to nourish a growing global population both sustainably and healthily, yet it requires our food system to change fundamentally, affecting practices in agriculture, food processing, -production, -distribution, and consumption [5].

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Omics technologies to investigate the in vivo neuroprotective capacity of natural extracts.

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Microalgae and food by-products are promising sources of neuroprotective molecules. Previous studies from our laboratory have demonstrated the *in vitro* neuroprotective activity of a *Dunaliella salina* microalga (DS) extract obtained with supercritical fluids [1], and an orange juice by-products (NAR) extract obtained by pressurized liquids [2]. The present work aims to evaluate the *in vivo* neuroprotective activity of these two extracts using an Alzheimer’s disease model based on a transgenic *Caenorhabditis elegans* strain (CL4176), which expresses the human Aβ1–42 protein. A time and dose dependent paralysis assay was performed, and the transcriptomics and metabolomics changes after the treatment were evaluated by RNA-Seq and GC/HPLC-MS technologies, respectively. The *in vivo* assay showed a dose-dependent protection against paralysis when the DS or NAR were used, being the paralysis of the worms reduced to 53% (for DS) and to 54% (for NAR) when 50 µg/mL of the extracts were used after 32 h of Aβ induction. At this concentration and after 26 h of paralysis induction, the transcriptomics analysis revealed the significant alteration of 150 genes (120 up-regulated and 30 down-regulated) after DS extract treatment, while 294 genes were altered by NAR extract (186 up-regulated and 108 down-regulated). Moreover, the combination of the different advanced analytical techniques allowed the identification of more than 750 intracellular metabolites, of which more than 60 were significantly altered by DS extract, while 36 were altered by NAR extract. The integration of these and previous results provides with new evidences on the neuroprotection mechanisms of these promising extracts, representing a step forward on the valorization of microalgae and food by-products as valuable sources of neuroprotective compounds.

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Microalgae Spirulina: a sustainable source of bioactive compounds with anti-inflammatory properties.

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Spirulina has been gaining popularity as good source of protein (63 g/100 g) and antioxidant compounds such as β-carotene and phycocyanin. Moreover, spirulina can be cultivated under specific conditions of light and pH with minimal environmental footprint. Phycocyanin, the most abundant pigment, has been found to have numerous health benefits, the most important one being the reduction of intestinal inflammation. It may, however, be crucial to investigate whether other bioactive molecules in spirulina can exert the same effect. The aim of this study was to assess the potential anti-inflammatory properties of three pigments previously extracted from spirulina: phycocyanin, pheophytin a, and pheophorbide a.

To this end, caco-2 cells, stimulated with interleukin-1β (IL-1β; 50 ng/ml) were used as a model of intestinal inflammation. The cells were supplemented with increasing concentrations of phycocyanin, pheophytin a and pheophorbide a (1, 5, 10, 25 and 50 μg/ml), for 1 hour and then exposed to IL-1β for 24 hours. The neutral red assay was used to evaluate the cytotoxicity of the compounds in the presence of IL-1β. The secretion of interleukin-8 (IL-8), an inflammation marker, was evaluated in the growth media via sandwich ELISA.

Although all pigments reduced il-8 secretion compared to the IL-1β-stimulated positive control (no supplementation), the most potent was pheophorbide a with 35-85% inhibition at the lowest-highest dose, compared with phycocyanin and pheophytin that reduced il-8 secretion at lowest dose by 25% and 16%, respectively.

Further analyses are underway to understand the mechanism(s) of action underpinning the anti-inflammatory activity and whether the same effect is maintained on differentiated intestinal cells.

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From agro-industrial by-products to dietary fibre-rich food ingredients: a "reactOmics" approach for their detailed chemical characterization.

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Nowadays, there is a growing interest in healthy nutrition and, consequently, dietary fibres. As there are high quantities of fibre-rich by-products from the agro-forestry industry, their reuse to produce new food ingredients is a viable option.

However, lignocellulosic by-products require the application of harsh conditions to extract valuable dietary fibre (hemicellulose fraction), in terms of temperature, which triggers many chemical reactions as the formation of oligosaccharides with potential functional properties, but also of a myriad of other molecules, mainly resulting from the degradation of sugars and phenols [1]. These compounds are not properly characterised and might have an impact in terms of toxicity in the final product. In this research, we tested hydrothermal treatment to extract hemicellulose from hazelnut shells. The composition of the extracts was investigated by a multiplatform 'reactOmics' approach, using several combined analytical techniques, namely GC-MS, ^{1}H-NMR, and UHPLC-IM-QTOF-MS. Commercial hemicellulose food supplements were also characterized with the same approach. The results showed an impressive diversification of oligosaccharides (target healthy compounds) in terms of their degree of polymerization, monosaccharides composition, and substitution, as well as many other molecules, mainly classifiable as phenols, furans, and degraded sugars, which should be studied in more detail for the safety of consumers.

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Outcomes of lactic acid fermentation on the phenolic content and in vitro bioactivity of mango seed.

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Mango (*Mangifera indica* Linn.) is a tropical fruit wide consumed which production was 57.1 million metric tons of mango in 2021. When processing for obtaining juice around 35-60% of the whole fruit is discarded as by-products (peels and seeds) that have reported several bioactivities attributed to phenolic compounds. Thus, the aim of this study was to investigate the fermentation of mango seed by different lactic acid bacteria (LAB) strains and its impact on phenolic composition and antioxidant and antidiabetic activities. LAB used were *Pediococcus acidilactici* CECT 5765T, *Pediococcus pentosaceus* CECT 923, two strains of *Lactiplantibacillus plantarum* (CECT 748T and 9567-C4), *Levilactobacillus brevis* CECT 5354 and *Lecuonostoc mesenteroides* CECT 219T. Phenolic compounds were determined by HPLC-ESI-TOF-MS, the antioxidant activity by DPPH and FRAP methods, and the antidiabetic activity by alpha-amylase inhibitory capacity. All lab strains exhibited good growth and good survival rates. It was revealed significant increases of 6 and 4% ($p < 0.05$) in phenolic compounds in seeds fermented by *Leu. mesenteroides* CECT 219T and *Le. brevis* CECT 5354, respectively, compared to non-fermented seeds. Phenolic acids were the major group of phenolic compounds accounting for 97% of the total phenolic content. Seeds fermented by *Leu. mesenteroides* CECT 219T and *La. plantarum* CECT 748T exhibited significant ($p < 0.05$) increments of 29 and 16%, respectively, in the sum of ellagic acid derivatives compared to control. Mangiferin was the major flavonoid detected and it achieved significant ($p < 0.05$) increases in the seeds fermented by *P. acidilactici* CECT 5765T and *P. pentosaceus* CECT 923,7 and 6%, respectively. Antioxidant and antidiabetic assays demonstrated strain and time-dependent variations with the highest increment in the seeds fermented by *P. acidilactici* CECT 5765T. Positive correlations were found between evaluated activities and phenolic compounds. The study highlights how carefully selecting bacteria can greatly influence the phenolic composition and its *in vitro* bioactivities in mango seed extracts. This process has the potential to enhance the recovery of phenolic compounds in mango seeds, offering exciting possibilities for their application in food and beverage processing.

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MAS and NMR spectroscopies for studies on Food authenticity and food content.

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Food analysis is incessantly requiring the development of more efficient, cost-effective, and robust method to investigate the quality and traceability of food commodities with respect to legislation and consumer demands.

Nuclear Magnetic Resonance (NMR) Spectroscopy is a powerful and well-established technology for detailed investigations of qualitative and quantitative characteristics of complex chemical and biological samples, like food, body fluids, or plant extracts. Yielding targeted quantification of single compounds as well as untargeted whole-matrix fingerprinting in a single run, NMR is specific and holistic likewise. Its reproducibility enables worldwide lab-to-lab spectra comparison and collective database build-up.

When coupled with uni- and multivariate statistical methods, a wealth of information can be extracted from NMR data, to generate classification, discrimination, and regression models.

This approach in particular supports complex mixture analysis on a Fourier-80 Benchtop NMR spectrometer, where the matrices' fingerprint details can be even more entangled. It can be shown – on an olive oil profiling example - that not only classification, but also quantification of single components or sum parameters by regression is possible at the benchtop NMR. Within this context, this study aims to elucidate the characterization and identification of stereochemical isomers associated with the health claim in Greek olive oil and evaluate the discrimination of olive oil samples from different agricultural backgrounds (variety, geographical origin). An innovative trapped ion mobility spectrometry (TIMS) coupled to ultra-high performance liquid chromatography–electrospray ionization quadrupole time of flight tandem mass spectrometry (UHPLC-QTOF) analytical method was developed for the analyses of 48 samples of Koroneiki variety from 3 different geographical origins in Greece (Peloponnese, Lesvos, Crete) and 33 samples of 5 different Greek varieties: Koroneiki, Kolovi, Adramytiani (Lesvos), Chiotiki (Chios), Thrumba (Samos). The discrimination studies, based on an untargeted approach, and Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) statistical models, showed the capability of TIMS-TOF technology to discriminate samples from different varieties and geographical origins, also combining the positive and negative polarity acquisitions. In particular, thanks to the TIMS activation, was possible to obtain the isomers separation (i.e. 5 different mobility peaks and MS/MS spectra were detected from 1 chromatographic peak) and isomer identification (applying a structure elucidation-aimed workflow). Therefore, an efficient geographical discrimination of the samples and a list of isomers as potential authenticity

Session 4 : Nutrient bioaccessibility and bioavailability. Do we need a new nutrition label?

The structure of the food matrix at different length scales drives the mechanism of digestion and the nutrient bioaccessibility and bioavailability.

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The food matrix structure is one of the key drivers to control the fate of food in the digestive tract and, consequently, the kinetics of nutrient release. Using in vitro and in vivo digestion models (pigs), we have demonstrated that, at identical composition, differences in dairy product macrostructure (liquid vs gel vs solid) lead to differences in gastric emptying, protein hydrolysis in the gut and amino acid bioavailability. When macrostructures are identical, differences at the microscopic scale can also have a strong impact on food digestion. For instance, egg white gels made by heat treatment at different pHs and ionic strengths exhibit differences in digestion kinetics depending on the ability of pepsin to diffuse into the egg white gel structure. Several examples of food matrix effect on the bioaccessibility/bioavailability of other nutrients (DHA, vitamins, minerals…) will be given. Correlation between bioaccessibility and bioavailability will be presented for different nutrients. The structure of food at different length scales can therefore be considered as a lever to control the kinetics of nutrients release during digestion and fulfil the nutritional needs of specific populations (elderly, obese, athletes).

The distinctive effect of different insect powders as meat extenders in beef burgers subjected to cooking and in vitro gastrointestinal digestion.

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To date, animal proteins from meat represent some of the main concerns related to the sustainability of food production[1]. In this scenario, partially replacing meat with non-meat substances with a high protein content confers interesting opportunities to reformulate meat products to make them more sustainable and promote human health[2]. In this regard, insects are described as a promising source of proteins; more importantly, at European level, there are currently four insects authorised by EFSA as novel foods, including *Tenebrio molitor* larva, *Locusta migratoria*, *Alphitobius diaperinus* larva, and *Acheta domesticus*. Before incorporating insect-based ingredients into different foods, it is important to fully evaluate potential food safety and food quality issues. In the meat science area, the most studied properties of edible insect proteins are their emulsifying capacity, together with stability, gel formation, water- and oil-retention capacity, and solubility[3]. However, some technological and acceptability issues exist mainly when considering the Western countries[4]. Despite the advances in meat products containing edible insects, their sensory, functional, and physicochemical properties and gastrointestinal behaviour remain poorly investigated. Therefore, in this work, mealworm (MWP), migratory locust (LP), and house cricket (CP) powders were tested as meat extenders at 5% inclusion (w/w) in beef burgers. According to scientific literature, the limit of incorporation of these new ingredients must not exceed 10% to avoid undesirable changes of meat properties. The untargeted metabolomics approach based on high-resolution mass spectrometry revealed different phenolic contents in the insect powders tested, recording the highest values in LP (1184.9 μg/g). The sensory analysis highlighted a higher visual and olfactory acceptability for MWP-burgers, followed by CP- and LP-burgers, whereas the texture of cooked burgers remained practically unaffected. Following a pan-cooking process, MWP-burgers and control samples exhibited comparable chemical profiles, while a significant down-accumulation of the heterocyclic amine 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline was observed in CP burgers. Additionally, the *in vitro* gastrointestinal digestion based on the harmonized INFOGEST protocol highlighted metabolomic trends similar to the control for MWP- and LP-burgers. Conversely, a reduced accumulation of lipids and increased content of dipeptides like glutaminylarginine (possibly acting as enzyme modulators) was observed for the CP-burgers. Therefore, our metabolomic findings provide new insights into the use of insect powders in meat reformulation.

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“Baby” fruits from pomegranate and prickly pear, underrated byproducts rich in gut bowel health-promoting compounds.

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Pomegranate (*Punica granatum* L.) and prickly pear (*Opuntia ficus indica* L. Mill.) are two of the most iconic fruit products cultivated in the Mediterranean area. A great interest in pomegranate has recently arose due to the peculiar biological properties of both its juice and side products that have in fact been extensively investigated, whilst other pomegranate by-products, such as those originating from the fruit thinning process, have not been so far studied in deep. Similarly, prickly pear fruits, cladodes and post-production side products are well known for their distinguished compositional features as well as biological and rheological attributes. No report exist in literature on the content, in terms of small-sized molecules and polysaccharides, of the flowers removed at the spring flush, practice realized to promote the growth and development of fruits of a late crop coming from the second bloom. To this end, immature pomegranate fruits from cultivar ‘Wonderful’ (Figure 1) and immature prickly pear fruits set from varieties red ‘Sanguigna’, white ‘Muscarella’ and yellow ‘Sulfarina’ were subjected to a targeted, innovative extraction procedure [1] to yield a series of matrices, which have been preliminary investigated and whose compositional characterization and biological validation is still undergoing [2].

Fig.1 *the immature pomegranate matrices, as whole fruits (A-B) and divided into peels and arils+ mesocarp /C-D) object of the study.*

[1] Laura Siracusa, Carmelo Drago, Giuseppe Ruberto, Giovanni Mario Pitari. IMMATURE POMEGRANATE EXTRACT FORMULATIONS (MBI17065-IT, depositato il 12/10/2020 e concesso il 26/10/2022 N. 102020000023920); PCT/IB2021/059338 (EU), CA3198627A1 (Canada), WO2022079592A (USA)

[2] Venera Russo, Alberto Continella, Carmelo Drago, Alessandra Gentile, Stefano La Malfa, Claudia Giovanna Leotta, Luana Pulvirenti, Giuseppe Ruberto, Giovanni Mario Pitari, Laura Siracusa. Secondary metabolic profiles and anticancer actions from fruit extracts of immature pomegranates. PLoS ONE 2021, 16, e0255831.

Evaluation of the effect of fractionation on seed-derived ingredients using a proteomic approach: a focus on antinutrients.

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The food production system contributes by one third to the global greenhouse gas (GHG) emissions with a relevant negative impact on the environment. Alternative protein ingredient, with high performing functional properties, are making their way on the EU market as sustainable alternatives to traditional egg and milk proteins. The use of whole flour or protein isolates from cereals, pulses and seeds is increasing in the food industry, with green pea predominating for its nutritional profile and performing functional properties as egg replaces. Ingredients production process can impact the amino acid quality and anti-nutritional factors.

In the H2020 EU-funded project Giant Leaps, ingredients produced with different technological approaches from pulses and cereals were characterised by advanced proteomics approaches for characterising the protein profile with a focus on of proteinaceous ANFs. The electrophoresis highlighted different proteomic profiles across technological processes, with the formation of aggregates and selective presence of specific bands as a function of the production process. The mass spectra were analysed using de-novo sequencing-based software to overcome the limiting number of curated protein sequencing for novel foods. According to these findings, protein quality, digestibility, and antinutrient levels are highly affected by extraction and/or fractionation methods.

This work was undertaken as part of the EU funded Giant Leaps project: Gap resolutIon in sAfety, NuTritional, alLergenicity and Environmental assessments to promote Alternative Protein utilization and the dietary Shift (Grant agreement ID: 101059632).

A snapshot of the metabolic cross-talk between the resident microbiota in ripening cheese inoculated with undesirable microorganisms.

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Raw milk cheeses harbor complex microbial communities. Some of these microorganisms are technologically essential, but undesirable microorganisms can also be present. While most of the microbial dynamics and cross-talking studies involving interaction between food-derived bacteria have been carried out on agar plates in laboratory-controlled conditions, the present study evaluated for the first time the metabolic cross-talk of resident microbiota and their changes of metabolite production directly in ripening raw milk cheese inoculated with *Listeria innocua*. Using a proxy of the pathogenic *Listeria monocytogenes*, we aimed at establishing the key microbiota players and their exchanged chemical signals in Latteria raw milk cheese over 60 days of ripening time.

The microbiota of both the control and *Listeria*-inoculated cheeses was analyzed using 16S rRNA targeted amplicon sequencing, while direct analysis in real time mass spectrometry (DART-HRMS) was applied to investigate the differences in the metabolic profiles of the cheeses. The diversity analysis and the taxonomic analysis showed the same microbial diversity trend in both the control cheese with the most representative genera being *Lactobacillu*s, *Streptococcus*, and *Flavobacteria*.

On the other hand, the metabolic fingerprints revealed that the complex interactions between resident microbiota were governed by continuously changing chemical signals. Changes in the amounts of small organic acids, hydroxyl fatty acids, and antimicrobial compounds, including pyroglutamic acid, hydroxy-isocaproic acid, malic acid, phenyllactic acid, and lactic acid, were observed over time in the *L. innocua*-inoculated cheese when compared with the control. The correlation of the taxonomic and metabolomics data showed that, in cheese inoculated with *L. innocua*, *Streptococcus* was significantly correlated with the volatile compounds carboxylbenzaldheyde and cyclohexanecarboxylic acid, while *Lactobacillus* was positively correlated with some volatile and flavor compounds (cyclohexanecarboxylic acid, pyroxidal acid, aminobenzoic acid, and vanillic acid). Using a multi-omics approach we demonstrated that the presence of undesirable microorganisms can lead to the changes in organic acids, flavor compounds and the subsequent production of antimicrobial molecules.

In vitro modulation of MetS colon microbiota by innovative and sustainable formulations of bakery products.

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The gastrointestinal tract and its microbiota play a pivotal role in human health, with dysbiosis—alterations in microbiota composition or function—linked to various disorders, including obesity, diabetes, systemic inflammation, and metabolic syndrome. While studies have established associations between gut microbiome and health/disease, causality remains unclear. Two key relationships need elucidation: a) the impact of gut microbiota dietary factors (prebiotics, probiotics, etc.) and b) disease-related factors (drugs, pathogens, lifestyle). *In vivo* studies, considered the gold standard, face challenges in terms of cost, time, ethical concerns, and differences between animal models and humans. Recognizing these limitations, *in vitro* models have emerged as a valuable tool for mode-of-action studies, supported by a European Commission survey (EURL ECVAM, 2021).

This research assesses the *in vitro* prebiotic potential of food prototypes developed by Dr. Schär company (Bolzano, Italy) in the form of savoury flatbread. The study employs the MICODE *in vitro* intestinal model, developed at the University of Bologna, simulating the colonic ecosystem with a fecal inoculum delivering human colon microbiota from metabolic syndrome (MetS) patients. Specific microbial taxa associated with MetS dysbiosis or improvements from dietary interventions are evaluated, including *Eubacteria*, *Firmicutes*, *Bacteroidetes, Bifidobacteriaceae*, *Collinsella aerofaciens*, *Desulfovibrio* spp., *Escherichia coli*, *Akkermansia muciniphila*, and others.

The food prototypes demonstrate *in vitro* potential to enhance ecological indicators related to dysbiosis in MetS patients' microbiota. While the extent varies among prototypes, a general positive impact on beneficial groups and their metabolites is observed, along with a negative effect on dysbiosis-associated groups, including a reduction in harmful metabolites like p-cresol and skatole. The study highlights the prototypes' ability to promote a eubiotic effect on the diet and mitigate dysbiosis-associated markers.

To gain a comprehensive understanding of microbial shifts resulting from the interaction with prototypes, further analysis using bacterial 16S RNA sequencing is anticipated. This will contribute valuable insights into the mechanisms underlying the in vitro effects of the prototypes on the gut microbiota, shedding light on their potential therapeutic applications for addressing dysbiosis-associated conditions such as MetS.

FoodOmics 2024

POSTERS' ABSTRACTS

Carob Syrup: prebiotic potential of a neglected functional beverage of Mediterranean countries.

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Carob syrup is a potential functional beverage obtained from the boiling of carob pods from the vigorous plant *Ceratonia siliqua* (L.). Carob is a perennial, evergreen plant, which in important, in the south of the Mediterranean countries, due to its environmental and economic significance. Carob has had an ancient use for its functional attributes in traditional nutrition and healthcare. Nowadays the syrup is neglected for functional

properties and is used as an alternative to cocoa in desserts or sweeteners. Carob syrup is a subject study of the PRIMA project GourMed, which aims to compare Mediterranean traditional foods produced and marketed following principles of fairness in the seek to exploit them to improve the involvement women in Tunisian rural economy. In this work three different carob syrups produced by Tunisian women were compared to two benchmarks available in the large distribution in Italy and Greece, for their prebiotic potential in comparison to FOS (fructo-oligosaccharides). Briefly, the prebiotic score, the bifidogenic activity and the volatilome (by SPME GC/MS) were characterized and their variables were correlated together and to some chemical physical parameters, as pH and Water Activity. From the obtained results, the growth of inoculated pathogenic *Escherichia coli* ATCC 25404 in carob syrup samples was lower in the fair Tunisian products, than in the Italian benchmark. The prebiotic score related to aero-tolerant probiotic *Lactiplantibacillus plantarum* NCIMB 8299 and the bifidogenic effect towards anaerobic-probiotic *Bifidobacterium bifidum* NCIMB 700795 gave results showing this trend of strength: FOS > Tunisian pilot products > Greek benchmark > Italian benchmark. From the volatilome of the samples, a matrix of around 40 normally distributed volatile compounds was constructed, characterized mainly by organic acids and aldehydes, described in higher contribution for the pilot products. When the prebiotic activity was correlated to the volatilome, significant positive correlations with short chain fatty acids and n-Hexadecanoic acid were found, while significant negative ones were found in respect to Furfural which is probably related to thermal process. In conclusion the artisanal Tunisian fair products were likely to have an higher prebiotic potential than the large distribution benchmarks. Such difference can be explained by raw material, formulation and process leading to a higher content and higher speciation of organic acids, that could have fostered more the growth of probiotics. Notwithstanding Furfural was a signature of the Tunisian products and its content should be taken under observation, as can result toxic for the consumer. The very next perspectives for this work are to enlarge the knowledge on carob syrup functionality towards the intestinal human microbiota with an *in vitro* intestinal model.

Biotechnological valorisation of chickpea flour for the production of value-added ingredients for innovative food formulations.

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Legumes are an excellent source of dietary proteins that could help to meet the increasing market demand for new and sustainable source of proteins. Chickpea (*Cicer arietinum* L.) is the second largest legume produced and consumed worldwide and it has a high protein content (17-22%) with good properties. The application of biotechnological processes on chickpea flour could improve its organoleptic, nutritional, and functional properties, making this matrix suitable to produce innovative food products (1). The aim of this study was to evaluate the characteristics of chickpea flour when subjected to fermentation using 5 species of yeasts and 6 species of lactic acid bacteria (LAB) for the selection of the most promising microbial strains to produce functional ingredient with high nutritional value. After assessing the capability of all the strains to grow in a mixture of chickpea flour and water (1:2 w/w) by plate counting method, samples were characterised for peptide (OPA assay), and sugars concentration (K-RAFGL 08/23, Megazyme), prebiotic activity, volatile molecule profiles (SPME/GC-MS), and sensorial acceptability (panel test). At the end of fermentation, the peptide content increased in all the fermented samples when compared to control, reaching the highest value in the sample fermented by *Lactococcus lactis* LBG2 (22 mg/g). On the other hand, glucose, sucrose, and stachyose content decreased in a strain/specie fashion. Some samples, especially those fermented by yeasts, showed prebiotic activity as they were able to promote the development of *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* subsp. *infantis* DSM 20088. Volatile molecule profiles of samples fermented with LAB and yeast strains presented a higher amount of acids (33.2 ppm of acetic acid in the sample fermented with *Lactobacillus paracasei* L compared to the control containing 0.14 ppm) and alcohols (94.7 ppm of ethanol in the sample fermented with *Saccharomyces cerevisiae* FB2 compared to the control containing 0.27 ppm), respectively. On the odour and visual evaluation, different perception and pleasantness were provided. Although further characterizations are required, these results indicate that microbial fermentation of chickpea flour is a promising tool to produce functional and flavouring ingredients that can be used in food formulation.

[1]. Sáez GD, Sabater C, Fara A, Zárate G. Fermentation of chickpea flour with selected lactic acid bacteria for improving its nutritional and functional properties. J Appl Microbiol. 2022 Jul 1;133(1):181–199.

Omics techniques for assessing the potential of high hydrostatic pressure to improve the shelf-life of fresh sausages.

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Fresh sausages are made by combining lean and fatty minced meat, often from pork, with salt and spices according to local recipes. The product has a short *shelf-life* (10–15 days) and must be refrigerated (0–4°C) due to its high pH (approx. 5.8) and high a_w (0.97 or higher). The primary factors causing the degradation of these products are the growth of the microorganisms in the raw material, resulting in the production of *off-flavor* and *off-odor*, the color changes caused by the myoglobin status, and the oxidative reaction brought on by the high fat content [1].

From a commercial point of view, fresh sausages' perishability and therefore short *shelf-life* are obstacles preventing these items from expanding their market share. Nonetheless, since heat treatment of the product is not feasible, the application of non-thermal treatments can be crucial. Among the latter, the use of High-Pressure Process (HPP) is particularly interesting. In this context, the purpose of this study was to assess the potential of different treatments of HPP in extending the shelf life of fresh sausages packed under vacuum and produced in a pilot plant. Treated and non-treated samples have been compared with regard their microbial population dynamics both with culture-dependent and independent methods (*i.e.* metagenomic analysis) to study the spoilage patterns in relation to the treatment applied. In addition, attention has been posed to the color of the sausages as an important product feature assuring the acceptability of consumers. These preliminary results showed promising potentiality of HHP in the maintenance of the safety and quality of non-fermented sausages, highlighting a possible role of this technique in the increasing of sustainability of the fresh sausages production chains, reducing food spoilage and wastes.

[1*]. Hugo, C. J., & Hugo, A. (2015). Current trends in natural preservatives for fresh sausage products. Trends in Food Science & Technology, 45(1), 12-23*

Microalgae Spirulina: a sustainable source of bioactive compounds with anti-inflammatory properties.

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Spirulina has been gaining popularity as good source of protein (63 g/100 g) and antioxidant compounds such as b-carotene and phycocyanin. Moreover, spirulina can be cultivated under specific conditions of light and pH with minimal environmental footprint. Phycocyanin, the most abundant pigment, has been found to have numerous health benefits, the most important one being the reduction of intestinal inflammation. It may, however, be crucial to investigate whether other bioactive molecules in spirulina can exert the same effect. The aim of this study was to assess the potential anti-inflammatory properties of three pigments previously extracted from spirulina: phycocyanin, pheophytin a, and pheophorbide a.

To this end, caco-2 cells, stimulated with interleukin-1b (IL-1b; 50 ng/ml) were used as a model of intestinal inflammation. The cells were supplemented with increasing concentrations of phycocyanin, pheophytin a and pheophorbide a (1, 5, 10, 25 and 50 mg/ml), for 1 hour and then exposed to IL-1b for 24 hours. The neutral red assay was used to evaluate the cytotoxicity of the compounds in the presence of IL-1b. The secretion of interleukin-8 (IL-8), an inflammation marker, was evaluated in the growth media via sandwich ELISA.

Although all pigments reduced il-8 secretion compared to the IL-1b-stimulated positive control (no supplementation), the most potent was pheophorbide a with 35-85% inhibition at the lowest-highest dose, compared with phycocyanin and pheophytin that reduced il-8 secretion at lowest dose by 25% and 16%, respectively.

Further analyses are underway to understand the mechanism(s) of action underpinning the anti-inflammatory activity and whether the same effect is maintained on differentiated intestinal cells.

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Combination of ultrasound technology and submerged fermentation for improving the phenolic content, antioxidant and antidiabetic activity of mango leaves extracts.

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Mango leaves are a by-product rich in phenolic compounds whose extract could be used for food, nutraceutical, and cosmeceutical applications. The objective of this study was to improve the extraction of phenolic compounds using two non-thermal approaches such as lactic acid bacteria fermentation and ultrasound technology via sonotrode. A Box-Behnken model and response surface methodology was used to optimize the sonotrode conditions to obtain high recovery of phenolic compounds. The optimal conditions established were 100% amplitude, 18 min and 45% ethanol/water. A total of 58 polar compounds were detected, 11 of them being tentatively identified for the first time in mango leaves. Fermentations were carried out with *Le. brevis* CECT 4121T, *Le. brevis* CECT 5354, *La. plantarum* CECT 748T and *La. plantarum* CECT 9567-

C4 for 24 h. This technique allowed major amounts of total phenolic compound between 22 and 53% with respect to the control, except for fermentation with *La. plantarum CECT 9567-C4*. The action of some enzymes and the conversion from bound state to a free state increased polar compounds and bio-transformed some of them. Thus, some compounds were hydrolyzed to simpler molecules like gallotannins. The antioxidant activity (measured by DPPH and FRAP assays) improved until a 9% after fermentation. Moreover, *Le. brevis* CECT 5354 could increase the alpha-amylase inhibition enhancing antidiabetic activity. In conclusion, submerged fermentation with lactic acid bacteria and ultrasound technology are valuable technologies able to produce extracts enriched in phenolic compounds from mango leaves.

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Influence of submerged lactic acid fermentation on phenolic content, antioxidant and antidiabetic activity of cherimoya leaves extracts.

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Cherimoya *(Annona cherimola Mill*.) is a fruit whose production and consumption generate add-value by-products like leaves rich in bioactive compounds. Cherimoya leaves have been used in traditional medicine for associated antioxidant, antidiabetic or anti-inflammatory activities. The goal of this study is to investigate the modifications in the profile of polar compounds resulting from fermentation with different strains of lactic acid bacteria. Thus, *P. acidilactici* CECT 5765T*, Le. brevis* CECT 5354*, La. plantarum* CECT 748T*, Leu. mesenteroides* CECT 219 and *P. pentosaceus* CECT 923 allowed to increment total phenolic compounds between 19.7% and 28.8% with respect to unfermented samples. This phenomenon could be produced by cell-wall breakage and conversion to a free state with segregated enzymes. Most of the phenolic compounds experienced notable increases after lactic acid bacteria fermentation. Among them, some were chlorogenic acid, procyanidin dimer type B, 5-*p*-coumaroylquinic acid,

calabricoside A, rutin or quercetin hexoside. *P. acidilactici* CECT 5765T got the best results for antioxidant and antidiabetic activity in comparison with control and other fermented cherimoya leaves samples. The compounds more correlated for both activities were *p*-coumaroylglucaric acid derivative, 5-*p*-coumaroylquinic acid, calabricoside A and rutin. In conclusion, submerged lactic acid bacteria fermentation of cherimoya leaves was effective for obtaining extracts rich in phenolic compounds with antioxidant and antidiabetic activities.

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Mediterranean and medicinal plants as source of bioactive essential oils for new natural functional ingredients.

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InnoSol4Med PRIMA-Section 2 project aims to increase the quality, safety, and nutritional value of traditional

Mediterranean foods using sustainable methods and innovative ingredients. Among them, essential oils (EOs) can have strong antimicrobial activity and can exert antioxidant actions, representing a feasible solution to meet the consumer demand for natural and healthy products and to reduce the environmental impacts of agro-food sector.

With the aim to test their bioactive potential, ten EOs obtained from Mediterranean and medicinal plants have been studied. Their composition has been analysed comparing the profiles obtained by two chromatographic techniques (SPME-GC-MS and direct injection in GC-MS of samples) and results showed interesting VOCs profile, with the presence of molecules that can exert antimicrobial function. In fact, the minimum inhibiting concentration (MIC) of EOs against some spoilage and food-borne pathogens has been assessed, highlighting promising results with oregano and cinnamon EO, showing MIC value of 250 mg/L against *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli*), probably due to their chemical composition. In addition, *in vitro* antioxidant potential has been assessed using different methods (FRAP, DPPH, and ORAC) highlighting promising potential for some EOs (*i.e*. cloves and cinnamon).

This research can contribute to increase the knowledge of these plant matrices that can be exploited as a source of natural and functional ingredients, characterized by bioactive compounds.

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Phenolic profile and the antioxidant potential of Allium species by-products.

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Onion peels, often discarded as waste, may hold untapped bioactive potential due to their rich content of bioactive compounds. This study aimed to investigate the phenolic profile and antioxidant potential of red and yellow onion peels through various assays. Onion peels were extracted using two different extraction methods, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE), using 50% ethanol. The total phenolic content (TPC) was determined using the Folin–Ciocalteu method, the phenolic profile using high-performance liquid chromatography (HPLC) and antioxidant activity was evaluated using diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and oxygen radical absorbing capacity (ORAC) assays. The results revealed a significant concentration of quercetin, epicatechin and protocatechuic acid in the onion peel extracts. The highest TPC (3789,58 ± 77,09 mg gallic acid equivalents (GAE)/L) was found for yellow onion peels extracted using UAE, while the

lowest (1602,08 ± 24,88 mg GAE/L) was found for yellow onion peels extracted using MAE. The inhibition of DPPH ranged from 81,31 ± 2,00 to 86,19 ± 0,66% for UAE extracts and 85,54 ± 0,63 to 87,45 ± 0,19% for MAE extracts. The higher reducing power was found for UAE extracts when compared to MAE ones, and the highest ORAC result was found for yellow onion peels extracted using UAE. In conclusion, onion peels exhibit notable antioxidant activity, suggesting their potential application as natural antioxidants in food and pharmaceutical industries. Further research into the different extraction methods and bioavailability of these antioxidants could contribute to the development of value-added products from onion peels, reducing waste and promoting sustainable utilization.

This research is supported by the PRIMA program under project InnoSol4Med (Project ID 1836). The PRIMA program is supported by the European Union.

The integrated machine learning and untargeted metabolomics approach revealed potential biomarkers related to authenticity and traceability of grated Parmigiano Reggiano PDO cheese.

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[4] Parmigiano Reggiano Cheese Consortium, Via J.F. Kennedy, 18, Reggio Emilia 42124, Italy.

Parmigiano Reggiano (PR) represents one of the most important Italian food products labeled with a Protected Designation of Origin (PDO), with its production chain regulated by applying production guidelines defined by the PR Consortium. Although it is made following strict production regulations, the chemical composition of PR cheese could be significantly affected by different factors across the dairy supply chain and the field of "foodomics" has increased our knowledge about the potential factors influencing the composition of cheese [1]. However, although the ability of metabolomics to inspect singular effects has been already confirmed in a limited number of samples [2], no comprehensive studies regarding the analysis of the multiple quality parameters on PR PDO products exist in the literature (despite this representing a realistic condition). In this work, a highly representative dataset of PR PDO grated cheeses (n = 60) was used to gain new insights into three main interacting factors involved in PR PDO cheese quality, namely ripening, altimetry of PR cheese production and rind inclusion level in grated cheese. Specifically, the novelty of this study was represented by integrating a novel deep learning tool, with a machine learning-based Random Forest (RF) algorithm used for classification purposes, and ANOVA multiblock OPLS (AMOPLS). Combining these two approaches allowed to shed light on factor contributions within the structured metabolomics data and to highlight potential distinctive markers related to the different altitude productions and rind inclusion levels in PR grated cheese.

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Alternative protein source from agricultural by-products: optimizing tomato seeds enzymatic digestion.

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In the present study, the need of alternative food protein sources meets the need to valorize and reduce agricultural by-products. The specific objective is the optimization of protein extraction from tomato seeds. The work fits into the framework of the AgriLoop project, a collaborative initiative that unites partners from Europe and China. The overall goal of AgriLoop is to enhance the value of agricultural production by upgrading underexploited residues, thereby contributing to a more sustainable and resource-efficient agricultural landscape.

The tomato seeds used in this study were provided by TomaPaint S.r.l. (Parma, Italy) and were separated from peels through a flotation process. The digestion process was carried out using seven different proteases and five different cell-wall hydrolytic enzymes, in order to find the most efficient ones. The digestates underwent through analysis, including the assessments of protein, total polyphenols and reducing sugars contents.

Preliminary results are promising, and based on these findings, a two-step digestion is planned. This approach involves the initial use of the most effective cell-wall hydrolytic enzymes, followed by digestion using the best protease. Once the best protocol is determined, it will be extended to other agricultural residues, such as tomato peels, whole tomato pomace, grape pomace and brewery spent grain.

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Predicting the solubility in Natural Deep Eutectic Solvents (NADES) using COSMOtherm: A study based in caffeic acid, syringic acid and β-sitosterol, three compounds of interest in date palm seeds.

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In last decades, novel green mixtures called Natural Deep Eutectic Solvents (NADES) have emerged as an alternative to conventional solvents such as ethanol or methanol for hydrophilic compounds, and hexane for hydrophobic compounds. Due to the possibility to modulate its polarity, NADES have been proven as excellent solvents in extracting compound mixtures (1). The tunable properties of the NADES allow for the creation of solvents optimized for specific application. In addition, the Conductor-like Screening Model software (COSMOtherm, BIOVIA, USA) has been used to screen a wide range of solvents, including NADES, reducing the number of experiments, time, and cost (2). The aim of this study was to use the COSMOtherm tool to predict the solubility of the compounds of interest in date palm seeds, namely, two hydroxybenzoic acids (caffeic and syringic acid) and a phytosterol (β-sitosterol) in five different NADES with varying molar ratios or water content. The activity coefficients of caffeic acid and syringic acid in NADES indicates that they are more soluble in Betaine:Urea (1:2) 75% and 50% compared to other NADES and to reference solvents. In contrast, β-sitosterol showed a superior solubility with Caprylic acid:Lauric acid, regardless of molar ratio, compared to other NADES and hexane. A heat map, to show the activity coefficients, and a figure based on the geometrical and energetical profile, through σ-surface as well as σ-profiles, of the solvents and solutes were created to illustrate the miscibility between NADES and target compounds.

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Results of an ultrasound-assisted extraction method for bioactive compounds from hot varieties of Tunisian paprika.

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Ultrasound-assisted extraction (UAE) allows the use of more environmentally friendly solvents and achieves higher extraction yields of biocompounds when compared with those obtained with conventional extraction techniques and solvents (1). The aim of this work was to obtain extracts rich with bioactive compounds from a Tunisian variety of paprika (*Capsicum annuum* L.), using Response Surface Methodology together with Box-Behnken design (2) to optimize UAE parameters (amplitude, sample-solvent ratio and time). Total phenolic compounds (TPC) (3), total carotenoids (TC) (4) and antioxidant capacity (DPPH) (5) were measured. Eight samples from different markets in Tunisia were pooled to obtain a standard sample. The optimal extraction conditions in a Q500 ultrasonic processor (Qsonica, USA) were 80% amplitude (400 W), 1/10 g/ml for the sample-solvent ratio, 80-20% ethanol-water (v/v) and 30 min extraction. The results showed values of 1292 mg GAE/100 g in TPC, 843 µg eq β-carotene/100 g in TC and 3.9 mM TE/100 g for DPPH. Values were within the range of other similar studies (6, 7). Based on these results, the extraction of bioactive compounds from hot paprika using an optimised UAE and 80% aqueous ethanol process is effective and can be considered as an alternative to other conventional means used so far, although further research is still needed on the characterization and application of the extracts.

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DNA can make the difference: the two sides of the entomological authentication of the honey.

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Honey is one of the most frauded animal-derived food products: the most common frauds are derived from the adulteration of the sugar components by intentional dilution with cheap syrups, and the masking and mislabelling of the geographic, botanical, and entomological origin of the honey. The entomological origin of the honey can be considered from two different perspectives: i) the subspecies of Apis mellifera that has produced the honey; ii) the footprints derived from plant-suckling insects that produce honeydew, which are always present in authentic honey. The declaration of the A. mellifera subspecies is relevant in some protected designation of origin (PDO) honey, providing an important link to boost the conservation and integrity of honey bee genetic resources. The identification of the A. mellifera subspecies can also provide an indirect information on the geographic origin of the honey, according to the natural geographic distribution of the different subspecies. Plant-suckling insects provide multidimension information related to the plant origin of the honeydew, according to the botanical specialization of some of these plant parasites, also providing indirectly information on the botanical and geographic origin of the honey. For the identification of the honey bee subspecies, we set up two assays to analyse DNA extracted from the honey and that targeted two regions of the A. mellifera mitochondrial DNA (mtDNA). The assays can distinguish between different subspecies carrying the mtDNA lineages A (e.g. A. m. siciliana), C1 (A. m. ligustica), C2 (A. m. carnica) and M (e.g. A. m. mellifera). Another assay that is able to identify the A. mellifera subspecies, was designed to genotype about 100 subspecies-informative honey bee single nucleotide polymorphisms (SNPs) using the DNA extracted from the honey and a genotyping-by-sequencing approach. The identification of plant-suckling insects is based on a targeted metabarcoding analysis on Rhynchota mtDNA conserved regions using next generation sequencing approaches, coupled with bioinformatic pipelines to interpret sequenced reads. These different methods are highly informative and are routinely applied in our lab to authenticate the honey based on their entomological origin.

Investigating genetic factors likely involved in the onset of broiler breast meat abnormalities: A focus on Collagen type IV.

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To investigate genetic patterns likely involved in the occurrence of white striping (WS) and wooden breast (WB) meat quality defects affecting fast-growing chickens (FG), a weighted gene co-expression network analysis (WGCNA) was performed. The analysis was carried out using the microarray data and phenotypic values significantly related to the occurrence of WS/WB defects (i.e., breast weight and height, and cooking losses; $P<.0001$) of 12 *Pectoralis major* muscles (PM) (6 affected by WS/WB vs. 6 normal) from FG broilers (42 days old). The analysis revealed genes encoding collagen type 4 (COL4) (*COL4A1* and *COL4A2*) as hub genes in the expression patterns significantly related to the traits considered ($P<.0001$). COL4 is one of the major components of the basement membrane of several tissues. Since abnormalities in COL4 structure were demonstrated to be involved in human myopathies [2], the coding region of the chicken *COL4A1* gene (specifically from exon 21 to 25) – was investigated. To this purpose, sanger sequencing of chicken cDNA samples belonging to both FG and medium-growing (MG) broilers (5 PM/genotype; 42 days old) has been carried out to assess potential differences in the *COL4A1* coding region between the two genotypes, considering MG as control group due to the lower incidence of WS and WB compared to FG. Results evidenced no differences between the two genotypes in the sequenced cDNA region of *COL4A1*. It could be speculated that, because of its biological importance, the *COL4A1* coding region is highly conserved among chicken genotypes, despite the different selection practices. Nonetheless, its involvement in the critical physiological events underlying WS and WB could not be entirely excluded.

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Cell culture models for assessing the effects of bioactive compounds in common buckwheat (Fagopyrum esculentum): a systematic review.

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Common buckwheat (CBW) is a pseudo-cereal that is grown and consumed worldwide. It is an excellent source of nutrients and is becoming increasingly popular as a functional meal when combined with other health-supporting factors. CBW and its by-products have various health benefits, making them ideal for functional food formulation and increasing their potential for agricultural, industrial, and pharmacological use. Although human studies are considered the gold standard for assessing the association between nutrition and health, credible in vitro/ex vivo models have allowed researchers to examine cellular and molecular pathways. Cell cultures are commonly used in clinical settings to develop model systems for studying fundamental cell biology, simulating disease mechanisms, or examining the toxicity or safety of substances. This paper presents a comprehensive evaluation of the biological effects of CBW supplementation on cell culture. Despite variations in experimental conditions across studies, CBW supplementation

consistently resulted in reduced cytokine secretion, oxidation products, cell signaling modulation, and hormone secretion. These effects were primarily attributed to the presence of polyphenols and the protein/peptide fraction. Although further research is needed, especially in vivo investigations, these findings suggest that CBW-based solutions could be highly beneficial in improving public health and quality of life.

Molecular features of proteins and starch in sprouted bean-fortified bread.

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Pulses are a worldwide component of human diet and play a crucial role in meeting protein requirements, particularly in developing countries. However, pulses contain antinutritional factors such as phytates and enzyme inhibitors. Currently, sprouting is gaining attention as a low-cost, sustainable, and effective way to increase both the levels of health-promoting components and nutrients' digestibility. This study explores changes in the protein pattern in wheat bread enriched with flour from 72-hours germinated cowpea (*Vigna unguiculata*), a time shown to be sufficient for almost complete breakdown of antinutritional factors. Bread was prepared by adding 25% of unsprouted or sprouted cowpea flour to wheat flour. Samples were characterized in terms of protein content and profile, and for the content in residual anti-nutritional factors (trypsin inhibitors and phytates), starch (total, slowly/rapidly digestible, resistant), and gut-fermenting oligosaccharides. A slight increase in total protein in bean-fortified breads was related to the incorporation of a legume-derived component with M_r around 45 kDa. A decrease in total, rapidly digestible, and total digestible starch was observed in bread enriched with either sprouted or unsprouted bean flour, whereas the content of resistant starch increased in samples containing flour from sprouted beans. The sprouted bean flour breads also exhibited the lowest levels of trypsin inhibitors and phytates, as expected. Bread samples were subjected to in vitro digestion following the INFOGEST protocol to evaluate protein breakdown at various digestion phases. Large and medium-sized proteins bands were no longer present in SDS-PAGE tracings of the supernatant of digests after the gastric phase. Duodenal digestion produced small-size peptides, not retained in SDS-PAGE gels. The nature of the protein hydrolysis products, their bioaccessibility, and the presence of potential bioactive species are current under study.

How to optimize the endowment of bioactive compounds in Nostrana di Brisighella monovarietal extra virgin olive oils.

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Olive oil, a cornerstone of the Mediterranean diet, has always been known for its excellent sensory, nutritional and health properties. Indeed, also based on a steady increase in demand for more sustainable food products and following ongoing climate changes, the olive oil supply chain is committed to a renewal of elements to improve its resilience and competitiveness in an increasingly globalized market. For these reasons, also the olive oil sector of Emilia-Romagna region (Italy) could gain even higher trust and appreciation by consumers proposing an extra virgin olive oil (EVOO) obtained through sustainable agricultural practices enhancing bioactive compounds. Thus, starting from a previous investigation [1], this research work focuses on different agronomic variables to optimize the endowment of the monovarietal Nostrana di Brisighella EVOO in its content in bioactive components (phenolic and positive volatile compounds) and sensory attributes (fruity, bitter, pungent and other positive notes), well-known to be associated with such of molecules. The agronomic variables considered (olives were obtained through integrated pest management or organic farming and harvested at four increasing maturity indices) influenced the compositional and sensory characteristics of the EVOOs produced with significant impact on the phenolic content related to the healthy properties ("olive oil polyphenols" health claim).

This research work is developed within the project funded under the PSR 2014-2020 program – Type of Operation 16.2 "INnovazione e PROmozione della filiera dell'OLIO extra vergine di oliva emiliano-romagnolo (INPRO-OLIO)”and within the project funded under the National Recovery and Resilience Plan (NRRP) - NextGenerationEU “ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods”.

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Influence of diet on human gut metabolome.

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Metabolomics contributes to dietary assessments by directing objective measures of different factors of human food consumption. Changes in dietary patterns lead to alterations in the microbiome, which is interlinked with the metabolome. The study was aimed to explore the effects of habitual dietary patterns and micronutrient supplementation on human gut metabolome and host health. NMR-based spectroscopy was used to analyse stool samples with a 500MHz spectrometer, at 298 K, using standard settings and processing at *noesypr1d* pulse sequence. A total of 150 features were identified in the faecal samples. Of these, 55 were annotated and quantified to known metabolites. The dietary analysis of patients was carried out using Nutritics application. The nutrient consumption and dietary assessment were scored into tertiles (T1= lowest consumption, T3= highest consumption). Kruskal-Wallis test revealed significant differences among the extreme tertile groups ($\chi^2= 6.90$, df=2, $p<0.05$). Dunn's post hoc comparisons carried out between the extreme tertiles showed that arabinose is significant with higher carbohydrate consumption ($p=0.03$); 2-hydroxy isovalerate, aspartate, methanol and nicotinate are significant with higher protein consumption ($p<0.03$); and malate is significant with higher fat consumption ($p<0.05$). In conclusion, results showed significant differences in human gut metabolome among extreme tertile groups with varying nutrient consumption. There are potential limitations in the study due to the small subject size. It requires further robust experimentation in the future.

Utilization of Agri-Food Industry By-Products as Sustainable Substrates for Pullulan Production by Aureobasidium Strains: Implications for Food Packaging Sustainability.

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Pullulan is a microbial exopolysaccharide obtained by fermentation of the yeast like fungus *Aureobasidium* spp. Pullulan has various applications as food additive in the form of a thickening agents and stabiliser. In the last decade, it has also been used for the formulation of edible coatings and films in food packaging. The production of pullulan by *Aureobasidium* spp. is strain dependent and is strongly affected by the growth medium and conditions. Due to the high value of pullulan, it is fundamental to find alternative low-cost substrates for its production. The food industry produces large quantities of waste and by-products and the possibility to use these substrates for the pullulan production by *Aureobasidium* represents an opportunity that need to be explored [1]. In this study, the capability of two different *Aureobasidium* strains (*A. pullulans var.melanogenum* DSM 2404 and *A. pullulans* DSM 3042) to grow and to produce pullulan in four different agri-food by products, was investigated. In particular, the *Aureobasidium* strains were grown on whey, olive pomace, grape pomace, and molasses. The pullulan production process was optimised taking into account different variables such as temperature, pH, substrate ratio and fermentation time. The results showed that the two tested *Aureobasidium* strains were able to produce pullulan in all the tested by-products. However, the production rate was strongly affected by the strain and the substrate. Both the strains showed the highest pullulan production on whey followed by olive pomace, grape pomace and molasses. Nonetheless, the strain *A. pullulans* DSM 3042 showed the highest pullulan rate in all the considered substrates and conditions. In conclusion, the utilisation of food industry by-products as a carbon source for pullulan production by *A. pullulans* strains is not only a sustainable approach but also has promising implications for the food packaging industry. Pullulan, known for its excellent film-forming properties, offers a biodegradable alternative for food packaging materials that meets the increasing demand for sustainable packaging solutions [2]. However, further studies in order to characterize the quality and purity of the pullulan obtained by the various by-product should be performed.

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High resolution analysis of multi-strain fermented foods by NGS using innovative molecular markers

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Analysis of the microbial composition of multi-strain fermented foods is a challenging task. NGS metabarcoding brought great advantages as fast analytical method to understand the microbial communities' composition for research purposes (1). However, its application in industrial settings for quality control and stability check, fermented foods and other products containing microbes, is very limited by the low biodiversity of species used (2) and by the lack of standard methods that can distinguish strains belonging to same species (3).

The newly developed method brings together fast and standardized approach of NGS metabarcoding with an improved accuracy in distinguishing close related species, subspecies and strains of the same species in a blend.

Our method relies on HPME markers (4) which are intergenic highly variable sequences flanked by conserved coding sequence that allows PCR primers design. The amplification, sequencing of HPME markers and matching with a manually curated qualified database, has been first validated on mock communities and then successfully applied for the identification of all species and subspecies in fermented foods.

HPME-based metabarcoding analysis allows to quickly detect and identify species and subspecies in a sample to confirm its composition reported in the label. According to species biodiversity and the presence of known signature sequences, it can also discriminate strains belonging to the same species. Moreover HPME-based metabarcoding can be coupled with standard 16S rRNA metabarcoding to reveal unexpected bacterial species and combined with flow-cytometry to provide relative quantification of each viable strain in the blend.

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Promoting Innovation of ferMENTed fOods (PIMENTO) - COST ACTION CA20128.

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The aim of the PIMENTO project is to foster innovation in Fermented Foods (FF) in order to maintain the Europe's leadership in this field. The PIMENTO project is going to stimulate the networking and knowledge transfer within the research communities and the industries, as well as to stimulate the debate with policy makers and consumers associations. PIMENTO is organized in 5 Working Groups (WG):

WG1 - Build a multi-actor operational network, building a common vision and collective engagement to the objectives;
WG2 - Cartography of fermented foods in the diet of COST Countries, highlighting the variability shaped through centuries;
WG3 - Health benefits and risks of fermented foods to foster their integration in nutritional public health strategies;
WG4 - Federating scientists and Fermented Food producers to boost innovation for society to facilitate and foster sustainable innovation;
WG5 - Dissemination, training & events for the long-term impact of the COST Action.

PIMENTO results and ongoing activities are:

WG2 is mapping and cataloguing FF in COST countries and have developed a questionnaire to investigate the frequency of consumption of FF in European countries;

WG3 is analysing scientific literature in order to make a meta-analysis of health benefits of FF and elaborate reports similar to those requested by EFSA in order to approve health claims;
WG4 is investigating bottlenecks of innovation in FF in order to elaborate a position paper addressed to the European Commission and national policy makers. Moreover, WG4 in elaborating a strategy to cluster all FF industries in order to increase critical mass and promote the sector.
The PIMENTO project is calling the research and the industry communities to collaborate in the ongoing work of the WG2, WG3 and WG4.

PIMENTO project website www.fermentedfoods.eu

Digestibility and protein quality of bread products made with insect flour.

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The use of insect proteins in food represents a burgeoning and innovative approach to address thegrowing challenges of global food security and sustainability. Insect proteins are rich in essential amino acids, vitamins, and minerals, making them a nutritious and sustainable option for human consumption. Beyond their nutritional value, insects are readily available and can be raised on organic waste products, contributing to waste reduction and creating a

circular economy. Therefore, adding insect flour to foods widely consumed, such as bread, is interesting to reach a large number of population. Therefore, in this work samples of bread made from processed insect flour and samples of bread made from commercial insect flour were studied and subjected to the simulation of gastrointestinal digestion to study the protein digestibility as well as the antioxidant effect after digestion to study its nutritional quality. The nutritional quality and the digestibility of a protein relates to the amino acids available for intestinal absorption that will meet the amino acid requirements for the various biological functions. For this purpose, a dynamic simulation of gastrointestinal digestion was carried out using the equipment designed by AINIA for each sample. The results obtained for the gastrointestinal digestion showed that the bread with processed insect meal had a higher digestibility results. To evaluate the possible antioxidant effect of breads made with insect flour, hepatocyte cell line (HepG2) was stressed with an oxidant reagent. Next, cells were treated with the soluble fraction from gastrointestinal digestion. ROS levels and gene expression biomarkers related with oxidation such as SOD1, CAT and GSS were evaluated. After treatment with the samples, no significant differences were observed both in ROS and gene biomarkers.

Exploring micro- and macromolecular properties in buckwheat couscous.

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Buckwheat is a pseudocereal particularly rich in functional and nutraceutical components, and a sustainable crop. However, buckwheat presents several anti-nutritional factors that limit nutrient bioavailability. Sprouting and fermentation - both environment-friendly and easy to scale up processes - may help in overcoming these drawbacks. This study reports on the overall micro- and macromolecular features of a buckwheat couscous enriched with sprouted buckwheat. Couscous was chosen as a natural, sustainable food, and the non-enriched product is already on the market.

The multidisciplinary approaches used in this study showed that sprouting induced changes in: 1) the proteins profile and the protein-protein interactions; 2) the ratio between soluble and insoluble polyphenols; 3) the content of many antinutritional compounds. The addition of sprouted flour to 50% did not impair the production of couscous that was similar - from a sensorial standpoint - to the non-enriched one. The couscous processing - that modifies the overall protein structure and the interaction among micro - and macromolecules in buckwheat flour - did not affect the positive modifications induced by sprouting, that were still present in the enriched product even

after cooking. The results from this investigation provide a comprehensive overview of the impact of processing - including sustainable processes - on the nutritional and biochemical attributes of buckwheat, offering valuable insights into the potential of this crop as a functional food.

Overcoming Matrix Effects in protein extraction to valorize the potential of agri-food by-products.

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Food chains generate a high volume of waste and byproducts, that have a relevant environmental and economic impact (1)(2). In this context, the valorization of agri-food-derived matrices represents a milestone in the circular economy philosophy, enabling in-depth studies of the molecular characteristics of residual proteins that could be tested through *in vitro/in vivo* approaches. However, the search for biological activities (e.g., biodefense or biostimulant) is strongly hampered by matrix effects due to macromolecular conformational changes and interactions occurring during food processing. Overcoming these challenges involves the application of diverse biochemical strategies, rooted in the chemical features of macro and micro-constituents, along with enzymatic treatments. These strategic approaches enable extensive characterizations of selected polypeptides or the utilization of “omic” methods.

In this work, we present some examples of strategies used for the molecular characterization of two by-products from food industry, namely okara from soybean and seeds from tomatoes. Our findings will allow the knowledge-based potential exploitation of these molecules obtained with innovative and mild technologies. Private and public companies operating in the nutraceutical, food packaging and agricultural fields and oriented to the implementation of new commercial applications may benefit from this approach.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

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Application of hydrophobic deep eutectic extracts obtained from orange by-products as packaging films.

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The food packaging sector is more focused on the production of more eco-friendly polysaccharides-based materials with functional ingredients (1). The aim of the present study was the formulation of chitosan (2% solution) films with hydrophobic natural deep eutectic (HDES) extracts obtained from orange peels (OP) by an optimazed heating-stirring extraction method. The extractions were obtained using lauric acid: octanoic acid (1:3) (C12:C8), octanoic acid: L-proline (4:1) (C8:Pro), and menthol: myristic acid (4:1) (Me:MA). Then, chitosan films were formulated with pure HDES and HDES OP extracts (10%, w/w) and characterized in terms of thickness, color, UV-Vis light protection, opacity, and biological properties. The antioxidant capacity was measured by the DPPH method and the antibacterial activity was evaluated by the agar plate diffusion assay against *Escherichia coli* and *Staphylococcus aureus.* Control film with chitosan was also evaluated, considering that chitosan by itself may have bioactivity (2). The films obtained with Me:MA were not considered for further evaluation due to their heterogeneous appearance. The addition of HDES OP extracts to chitosan films significantly increase the thinckness, opacity and the yellow tone (higher values of yellowness, b*, and lower Hue angle values). The films enriched with C8:Pro OP extract and C12:C8 OP extract reduced the UV light more than control film and the films enriched with pure HDES. Then, the light transmittance of the control film was 85%, while the maximum transmittance value for the enriched films was 30%. The control chitosan films exhibited a low antioxidant activity of 19.12 ± 0.30 µg/AAE compared to the film enriched with C8:Pro OP extract (75.03 ± 0.50 µg/AAE). Films enriched with C12:C8, pure HDES and OP extracts, exerted antimicrobial activity against just *S. aureus* and the films with C8:Pro OP extracts against both bacteria. These results suggest that chitosan-enriched films could be an interesting material for the preparation of active food packaging.

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Hydrophobic Natural Deep Eutectic Solvents for the extraction of carotenoids from date palm seeds powder.

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Studies have shown that the waste products from dates such as the seeds contain ample amounts of biocompounds, such as carotenoids (1). Green solvents, “Natural Deep Eutectic Solvents” (NADES), have been used as a new way to extract phytochemicals in a sustainable and environmentally safe way (2). The aim of the present study is to analyze the quantity of carotenoids obtained with two hydrophobic NADES in date seed powder (DSP). The extraction was studied by heating-stirring for 30 minutes, at a temperature of 45±5 ºC, using a ratio (solid-liquid, w/v) of 1/3, 1/6 and 1/9 and two hydrophobic NADES, octanoic acid:L-proline (2:0.5) (Oct:Pro) and octanoic acid:lauric acid (3:1) (Oct:Lau) and also a control extract, hexane (Hex). Prior to extraction was made, date seeds were dried and powdered with a grinder. The carotenoids were extracted using the method of Leite *et al*., 2021 (2). Results have shown that, for the 1/3 ratio, there have been significant differences between Hex and Oct:Pro (0.03 vs 4.7 mg carotenoids /100g date seed powder), Hex and Oct:Lau (0.03 vs 0.4 mg carotenoids / 100 g DSP)) and Oct:Pro and Oct:Lau (4.7 vs 0.4 mg carotenoids / 100 g DSP). In the case of the 1/6 ratio, results showed that the sample that had the most amount of carotenoids were Oct:Pro (4.1 mg carotenoids / 100 g DSP), followed by Oct:Lau (0.3 mg carotenoids / 100 g DSP) and Hex (0.05 mg carotenoids / 100 g DSP). Finally, in the case of the 1/9 ratio, there were significant differences between all the samples but, as with the previous ratios, the sample that has extracted more has been Oct:Pro (2.9 mg carotenoids / 100 g DSP), followed by Oct:Lau (0.5 mg carotenoids / 100 g DSP) and hex (0.05 mg BCE / 100 g DSP). Overall, this indicates that most powerful solvent to extract carotenoids is Oct:Pro, followed by Oct:Lau and Hex.

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^{1}H-NMR-based metabolomic profiles of zucchini (Cucurbita pepo L.) grown with different agricultural practices.

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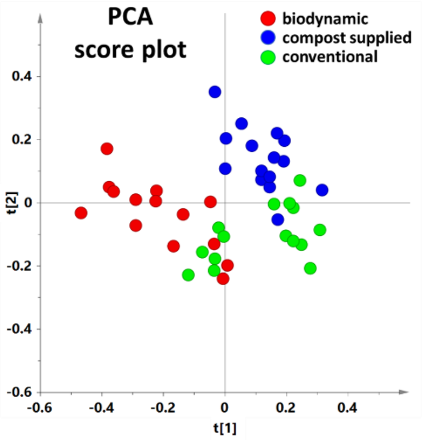

Zucchini (*Cucurbita pepo* subsp. *pepo*) is a seasonal vegetable (courgette) characterized by health properties due to the content of several bioactive molecules such as phenolic compounds and organic acids [1]. For this reason, the consumption of zucchini, and other seasonal vegetables, is highly recommended, especially as a part of the Mediterranean Diet. The aim of this study was to evaluate using a ^{1}H-NMR based metabolomics approach [2] the effect of different agricultural practices on the zucchini organoleptic and nutritional properties. A specific investigation on the zucchini juice obtained from three different cultivations (biodynamic, compost supplied, conventional) of the same cultivar *Vitulia* (Syngenta CV 2832) was performed by ^{1}H-NMR spectroscopy and Multivariate Analysis (MVA). The obtained results demonstrated a clear sample clustering according to the different used agricultural practices and allowed highlighting interesting differences in the sugar (higher for compost supplied and conventional samples) and amino acids (higher for biodynamic samples) relative content among the observed groups.

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1H NMR metabolic profiling for the geographical origin characterization of the Apulian EVOOs.

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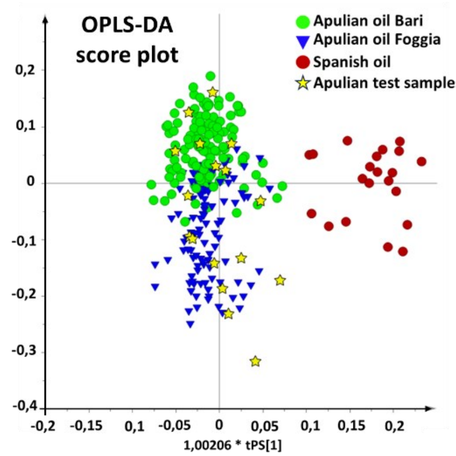

The geographical origin assessment of extra virgin olive oil (EVOO) is widely recognized as a factor that increases consumer awareness of the product authenticity. In several countries, labelling requirements and/or current legislation require the declaration of geographical origin. On the other hand, currently, despite considerable research efforts in the field, official methods to evaluate the origin of EVOO have not yet been established [1]. We have recently been involved in a project, aimed at building a ^{1}HNMR metabolic profiling database of Apulian EVOOs, for geographical origin assessment purposes. EVOOs obtained by both standard and laboratory milling, using olives originating from specific areas of the Apulia Region (the major EVOO producer in Italy), were considered. The relevant cultivars currently in production and included in the Apulian PDOs were selected. These included: PDO “Terra di Bari” (Coratina, Ogliarola barese, Cima di Mola); PDO “Collina di Brindisi” (Ogliarola, Cellina di Nardò, Leccino); PDO “Dauno” (Peranzana, Ogliarola garganica, Coratina); PDO “Terra d’Otranto” (Cellina di Nardò, Ogliarola salentina). The obtained database profitably allows a characterization of the Apulian EVOOs and their differentiation from other national and international EVOOs.

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CIPROMED project: Circular and Inclusive utilization of alternative PROteins in the MEDiterranean value chains.

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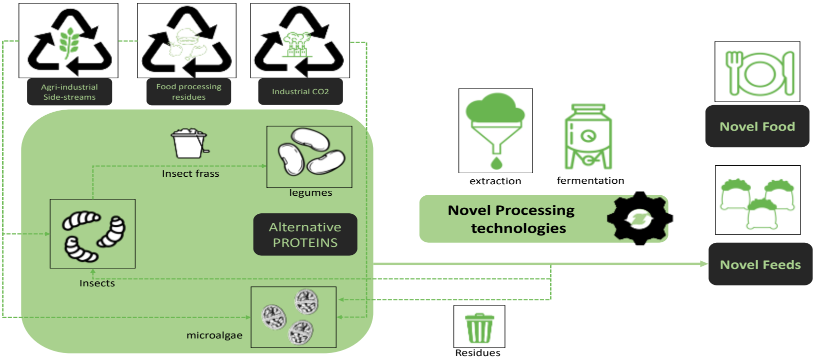

The main objective of the CIPROMED (https://cipromed-project.com/) project is to enhance the stability and resilience of agri-food production systems in the Mediterranean region. This will be achieved by directly utilizing locally grown traditional crops and extracting proteins from agri-industrial side-streams. The project will also focus on transforming the residues left after protein extraction into valuable proteins sourced from insects, legumes, microalgae, and fermentation products. Specifically in the food sector CIPROMED will: 1) create protocols for the production of new protein value chains using high protein agricultural crops, insects, microalgae, and agri-industrial side-streams; 2) evaluate protocols for insect rearing and cultivation of heterotrophic and autotrophic microalgae; 3) develop advanced extraction, stabilization, and technological conversion methods to obtain protein ingredients; 4) characterize the sensory, nutritional, technological, functional, as well as safety and anti-nutritive factors of the proteins; 5) formulate and evaluate new food products; 6) assess the environmental impact and economic implications of the new products and technologies; 7) understand public perceptions and beliefs regarding the novel protein production; 8) update the existing EU regulation framework on the use of novel proteins (Novel Foods).

CIPROMED financial support has been provided by PRIMA, a program supported by the EU under GA No. 2231 (PRIMA Call 2022 Section 1 Agri-food IA)

Metabolomics evaluation of invasive species in the Mediterranean Sea for possible commercial purposes.

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The Mediterranean Sea is well known as one of the most invaded by non-autochthonous species in the world [1]. Exploiting introduced species for alimentary purposes could serve as an effective strategy for mitigation and control, turning an environmental challenge into an opportunity. This investigation (UseIT project) focused on a metabolomic assessment of two invasive bivalves of the genus *Anadara* (*A. kagoscimensis* and *A. transversa*) from the Adriatic Sea and three non-indigenous fish (*Siganus luridus*, *Siganus rivulatus*, and *Pterois miles*) sampled from both Cyprus and Crete. Nuclear magnetic resonance (NMR) and multivariate statistical analysis

(PCA, OPLS-DA) were used for the metabolomics profiling of the aqueous and lipid extracts [2] from tissues of 60 samples. The results revealed significant differences in metabolic profiles of the two examined *Anadara* species as well as between *Siganus luridus*, *Siganus rivulatus*. Geographical variations of metabolic profiles were also observed for the *Siganus* and *Pterois miles* populations of Crete and Cyprus, which are likely the result of different environmental factors.

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UseIT, "Using Operational Synergies for the Integrated Management of Invasive Alien Species in Italy" project (Research Projects @CNR, carried out in collaboration with the IRET CNR and IAS CNR Institutes.). Marina Chiappa and Matilde Cella for samples collection.

Betaine-based Natural Deep Eutectic Solvents for a suitable and green extraction of polyphenols in date palm seeds powder.

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Date palm seeds are a waste product from the food industry, rich in bioactive compounds, such as polyphenols (1). In recent years, Natural Deep Eutectic Solvents (NADES) have been found to be a good alternative to conventional solvents. The aim of the present study is to compare the amount of polyphenols in different date seed extracts obtained with hydrophilic NADES.

Therefore, the extraction capacity of phenolic compounds has been studied doing heating-stirring method for 45 minutes, in a temperature of 45±5 ºC, using a ratio (solid-liquid, w/v) of 1/3, 1/6 and 1/9. After drying, date seed was powdered with a grinder. The analysis of total polyphenols content (TPC) (2) was carried out comparing two hydrophilic NADES, in a specific molar ratio, betaine:urea (1:2) (Bet:U) and betaine:glycerol:glucose (4:20:1) (Bet:Gly:Glu), with an Ethanol-H2O, 50% v/v, (EtOH 50%) as a control extract. In all instances, 1/3 ratio shown the highest amount of polyphenols. It has been observed that there are significant differences in the amount of polyphenol extraction at 1/3 ratio, between the control and Bet:U (72.3 vs 89.4 mg GAE /100 mL extract) and between Bet:U and Bet:Gly:Glu (89.4 vs 69.5 mg GAE / 100 mL extract), but not with control and Bet:Gly:Glu (72.3 vs 69.5 mg GAE / 100 mL de extract). This result was also observed for 1/9 ratio. In the case of extracts with a 1/6 ratio, it was observed that there were significant differences between all solvents: EtOH 50 % and Bet:U (27.9 vs 45.2 mg GAE / 100 ml extract); EtOH 50 % and Bet:Gly:Glu (27.9 vs 37.6 mg GAE / 100 mL extract), and between Bet:U and Bet:Gly:Glu (45.2 vs 37.6 mg GAE / 100 mL extract). Bet:U could be an alternative solvent for the extraction of polyphenols in data seed powder.

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Multi-analytical characterization of Parmigiano Reggiano PDO cheeses produced with milk from different dairy cows feeding.

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This investigation is addressed to characterize Parmigiano Reggiano PDO cheeses produced with milk from dairy cows fed different forage sources. In particular, ten samples of

Parmigiano Reggiano PDO cheeses, seasoned for 24 months, were produced with milk from dairy cows fed dry hay (PDH; N=5) and fresh forage (PFF; N=5). Instrumental volatilomics technologies (HS-Flash GC-FID and HS-GC-IMS), texture and image analyses, and sensory descriptive analyses (QDA®) were carried out. To investigate possible relations between dairy cows diet and cheeses microbiome and to assess samples safety, microbiological analyses were performed on total mesophilic bacteria (TMB), *Lactobacillus* spp., *Lactococcus* spp., yeast and molds, *Staphylococcus* spp. (including *S. aureus*), total Enterococci, and *Pseudomonas* spp. Instrumental, microbiological and sensory data were jointly elaborated. The results highlighted visual differences between the two experimental groups: indeed, the Parmigiano Reggiano cheeses belonging to the PFF group showed a higher intensity of yellow than PDH for both sensory and image analyses. Instrumental and sensory results were also discussed to investigate olfactory, taste, texture, and visual characteristics. In addition, all samples fulfilled the microbiological safety parameters and showed a similar TMB cell load. Such a multi-instrumental, microbiological and sensory characterization allows to obtain a unique fingerprinting analytical profile for each Parmigiano Reggiano PDO cheese, produced with milk from dairy cows fed different forage sources, thus contributing to the quality and authenticity control of this typical high-value food product.

This research was supported by AlmaIdea 2022 research project "INARIM - Indagini analitiche rapide dell'aroma e di immagine su prodotti alimentari di origine animale" funded by European Union - NextGenerationEU, by EU H2020 research project "INTAQT - INnovative Tools for Assessment and Authentication of chicken meat, beef and dairy products' QualiTies" (G.A. n. 101000250) and by

National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 “ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods”. This manuscript reflects only the authors’ views and opinions, neither the European Union nor the European Commission can be considered responsible for them. The authors gratefully acknowledge Dr. Mattia Marmiroli, Dr. Marco Nocetti, and the “Consorzio del Formaggio Parmigiano Reggiano” for providing the samples.

The addition of polysaccharide gums to Aronia melanocarpa purees modulates the bioaccessibility of phenolic compounds and gut microbiota: A multiomics data fusion approach following in vitro digestion and fermentation.

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This study aimed to determine how the addition of gellan, guar, locust bean, and xanthan gums affected the polyphenol profile of *Aronia melanocarpa* puree and the human gut microbiota after in vitro gastrointestinal digestion and large intestine fermentation (1). The different gums distinctively affected the content and bioaccessibility of phenolics in Aronia puree, as outlined by untargeted metabolomics. The addition of locust bean gum increased the levels of low-molecular-weight phenolics and phenolic acids after digestion. Gellan and guar gums enhanced phenolic acids' bioaccessibility after fermentation. Interactions between digestion products and fecal bacteria altered the composition of the microbiota, with the greatest impact of xanthan. Locust bean gum promoted the accumulation of different taxa with health-promoting properties. Our findings shed light on the added-value properties of commercial gums as food additives, promoting a distinctive increase of polyphenol bioaccessibility and shifting the gut microbiota distribution, depending on their composition and structural features.

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Fair Emmer flour differently affects prebiotic potential of bakery and pasta food products.

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To ensure or aim at food quality, profitability, and sustainability, the GourMed PRIMA project aims to provide new, innovation embedding, optimized governance and operational models of agri-food supply chains. It also aims to balance power and value appropriation among Mediterranean value chain actors and enhance value creation by Mediterranean value chains (regional vs. large distribution). Under the aegis of the PRIMA project GourMed, different food products formulated principally with Emmer were assessed for their prebiotic potential in comparison to FOS (fructo-oligosaccharides). The Emmer products were obtained from Prometeo SRL (Urbino), a company participating to the project as pilot study, selected for the fair procedures that regulate its production chain, and were compared to similar large distribution products, defined as the benchmarks. In particular, Emmer flour was employed to produce two types of products, i.e. pasta and bakery products (taralli) to understand what process marks more the Emmer benefits in comparison to the corresponding largely distributed benchmark. Briefly, the growth of intestinal pathogen, the prebiotic score and the bifidogenic activity were characterized by a microbiomics approach. and their variables were correlated together and to values of proximate recipes.

Considering pasta, the growth of pathogenic *Escherichia coli* ATCC 25404 and that of aerobic probiotic *Lactiplantibacillus plantarum* NCIMB 8299 were similar between fair and benchmark products ($p > 0.05$). Otherwise, the pilot products fostered the growth of *Bifidobacterium bifidum* NCIMB 700795 of an extent of 3.4 Log units of mean, significantly differing to benchmarks ($p < 0.05$), but not to FOS ($p > 0.05$). For pasta, no prebiotic score towards *L. plantarum* was measured, but the

bifidogenic effect was approximately three times higher in the pilot products ($p < 0.05$).
Considering “taralli”, clearer results were obtained, confirmed by the potent prebiotic score and bifidogenic effect similar to FOS. In particular, for the prebiotic score no differences were found between pilot and benchmark products (p = 0.80), nor between the products and FOS (p = 0.32 and p = 0.10, for fair and benchmarks, respectively). Interestingly, the bifidogenic effect has shown different performances between products. In detail, the bifidogenic effect of pilot product was significantly higher than that of the benchmark (p = 0.03) and similar to that of FOS (p = 0.08). Further studies are required, to explain the mechanism under this effect, and to enlarge investigation on in vitro and in vivo functionalities towards intestinal microbes.

Production of volatile compounds from fermented clementine by-products by yeasts and lactic acid bacteria.

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The circular bioeconomy approach represents an opportunity to address environmental and economic issues in a sustainable and innovative way. In this context, the development of food by-products valorisation strategies based on tailored biotechnological processes perfectly fits with the circular bioeconomy framework. Microbial fermentation by selected GRAS microbial species can improve the functional, qualitative, and sensory properties of the fermented substrate. The aim of this study was to investigate the contribution of different microorganisms (yeasts and lactic acid bacteria) to the production of volatile aroma compounds starting from agro-industrial residues such as the clementine juice processing by-products. More than 185 compounds relevant for the food sector were detected through HS-SPME-GC-MS analysis. The most prevalent substances were the terpenes, including linalool and alpha pinene. It is well-known that terpenoids are linked to citrus fruit peels and their essential oils which are valuable compounds to the food and fragrance industries. Moreover, several aldehydes, alcohols, and esters, which are identified as biomarkers for discriminating among different citrus varieties by affecting their typical odor and sensory properties, were associated with the fermented clementine by-products to different extents in relation to the microbial starter used for the fermentation. These compounds are responsible for flavours which are generally appreciated such as floral, citrus, sweety and minty. In conclusion, metabolites derived from microbial fermentation can improve the sensory profile of by-products thus ensuring their valorisation into flavouring ingredients for the food industry.

Synthesis of a phenolipid and evaluation of its antiproliferative activity: a first overview on the hydroxytyrosyl-punicate.

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Phenolic compounds are one of the major classes of bioactive compounds. In spite of their beneficial health effects, these compounds could present problems of solubility and absorption or they could interact with macromolecules in biological systems, compromising their bioactivity. Struggling

for a solution, the research has focused on the synthesis of antioxidants with amphiphilic properties, thereby obtaining so called phenolipids, which are obtained by the esterification of lipids and phenolic compounds. Hydroxytyrosol (HT) is one of the most studied natural phenolic compounds that has shown a wide bioactivity. Growing attention has been paid on its lipophilic derivatives with omega-3, -6 and -9 polyunsaturated fatty acids because of their health promoting effects.
On behalf of this premise, the aim of this work was to synthesize a novel phenolipid formed by HT and punicic acid to reinforce their potential towards anti-cancer activities in breast and lung cancer cell lines (MDA-MB-231 and A549, respectively). The hydroxytyrosyl-punicate (HT-PA) showed significant activity against the non-small cell lung carcinoma A549 cells being more active than HT and PA.

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Evaluation of lactic acid fermentation on the phenolic content and antioxidant and antidiabetic activities of three genotypes of olive leaves.

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Olive leaves represent about 10% of the total biomass of olives collected for the production of olive oil and around 25 kg of leaves are lost per olive tree during tree pruning. They have been previously reported to have several bioactivities attributed to its phenolic content. Thus, the aim of this study was to evaluate the influence of the fermentation of olive leaves ('Arbequina', 'Picual' and 'Sikitita') using 6 different lactic acid bacteria (LAB) strains on the obtention of enriched extracts on phenolic compounds with high antioxidant and antidiabetic activities.

Most of lab strains exhibited good growth and good survival rates. Regarding total phenolic compounds, *P. acidilactici* 5765T at 24h and 48h fermentation were the treatments that allowed a significant increase of these compounds compared to control in all cultivars (11.7-21.4%). Indeed, *Leu. mesenteroides* 219T also produced an increment of total phenolics in 'Picual' and 'Sikitita' (8.1% and 28.7%, respectively). Fermentations also allowed the increment of hydroxytyrosol derivatives; different behaviors were observed depending on the cultivar, but *La.*

plantarum 9567-C4 gave caused to an increase of hydroxytyrosol derivatives in the 3 cultivars, being more pronounced in Picual (36.5%). Elenolic acid increased in all cultivars after fermentations using *P. acidilactici* and *Le. brevis 5354* (increments of 20.4-47.4%).

Total phenolic compounds were significantly correlated with DPPH and FRAP in the 3 cultivars as exception of FRAP in 'Arbequina'. Finally, antidiabetic activity also improved after fermentation in the 3 cultivars, being *P. acidilactici* 5765T and *Pe. pentosaceus* CECT 923 the strains that produced better results in 'Picual' and 'Sikitita' and *La. plantarum* CECT 748T and 9567-C4, the best ones for 'Arbequina'.

Thus, submerged fermentation could be proposed as an olive leaves treatment aiming the increase of hydroxytyrosol, elenolic acid derivatives and total phenolic compounds concentration, as well as antidiabetic activity.

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Polyphenolic content and sensory profile assessed by e-nose and e-tongue of an orange juice fortified with polyphenol-enriched extracts using Natural Deep Eutectic Solvents.

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Natural deep eutectic solvents (NADES) are new green and environmentally friendly solvents used as an alternative to conventional organic solvents[1]. NADES are non-toxic and biodegradable[2], which makes them suitable to be used in the food industry. The present study aimed to evaluate the sensory profile and the polyphenol content of orange juices fortified with orange peel NADES extracts at different concentrations. Four choline chloride-based NADES were mixed with fructose (ChChl:Fruc), glycerol (ChChl:Gly), and glycerol:citric acid (ChChl:Gly:CA) and used for the extraction of polyphenols from orange by-products. Fresh orange juice (OJ) was obtained by pressing and polyphenol-enriched extracts were added to the OJ at different percentages (10 – 50 %). The sensory profile was evaluated by e-tongue and e-nose analysis while total phenol content (TPC) by spectrophotometric techniques. Results from e-nose and e-tongue showed that 15% and 30% of supplementation represented the highest concentration without significantly deviating from pure OJ, respectively. For this reason, the 15% concentration of extracts was selected for subsequent determinations of total phenolic content, which was evidenced as ChChl:Fruc, ChChl:Xyl and ChChl:Gly:CA, but ChChl:Gly, provided 160% more TPC than the pure OJ. Although

further studies are needed to define the microbiological stability and bioaccessibility of phenolic compounds, NADES may represent a green alternative to organic solvents for the extraction of phenolic compounds from orange by-products as well as for the formulation of new polyphenol-enriched food products.

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Impact of process conditions on probiotic survival and physicochemical characteristics of fermented milk containing functional vaginal strains.

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Functional foods are considered a promising dietary strategy to reduce incidence of human illness thanks to their active components [1]. The project FUNPRO-L wants to develop a probiotic fermented milk designed to be good, from a sensory perspective, healthy and functional since made with exopolysaccharides-producing lactic acid bacteria and vaginal probiotics. First, acidification kinetics were determined by applying 1 or 3U of the commercial starter Lyofast ST440 (Sacco) in skimmed milk incubated at 45°C for up to 24h. 4.5 and 7h of fermentation already dropped the pH to around 5 and 4.5, respectively. Therefore, these two incubation times and 1U of the starter culture were independently tested with 5 different vaginal probiotics (*Lactobacillus crispatus* BC1, BC4 and BC5; *Lactobacillus gasseri* BC9 and BC12), previously characterized for their functional properties [2,3], supplemented at 7 and 8 Log CFU/ml. pH, starter culture and probiotics viability, water holding capacity (WHC), and volatile molecule profiles of the fermented products were evaluated after 0 and 28 days of storage at 5°C. After 28 days of storage, control samples which underwent to 7 and 4.5h of fermentation showed a pH of 4.0 and 4.5, respectively. Addition of vaginal strains, especially at 8 Log CFU/ml, determined a further reduction of the pH up to 3.84 (7h) and 4.07 (4.5h) with BC5 and BC9, respectively. A drop of about 1 and 0.5 Log CFU/ml of the probiotics and the starter culture were observed in all the samples fermented for 7h and 4.5h, respectively. WHC increased after fermentation with vaginal strains, while it was maintained or reduced upon 28 days of storage, depending on the probiotic applied. The analyses of the volatile compounds showed specific fingerprints for each strain and concentration used. This preliminary screening provided the basis for the production and formulation of a fermented milk, containing functional probiotics, intended to promote female well-being.

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Tailored microbial starters to valorise clementine residue into functional ingredients.

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Citrus juice production generates significant residues and wastes with high-value compounds, which can be valued by the food industry rather than being discharged, used for energy production or animal feeding. The overall aim of this study was to set up a tailored biotechnological process, based on a non-thermal pre-treatment and microbial fermentation with GRAS microbial species to produce a functional ingredient from clementine by-products (BPs). Since the chemico-physical features of the citrus BPs can inhibit microbial growth, a preliminary screening of 40 strains of different microbial species among yeasts and lactic acid bacteria (LAB) was carried out for their growth ability and for the impacts on total phenolic content (TPC), antioxidant activity and sensory profile of the fermented BPs. Results showed that some of the tested yeasts grew after 24-48 hours, while others presented a prolonged lag phase and growth was detected only after 5 days (overall mean cell increases of 2-3 log CFU/g). All the LAB were able to adapt and survive to the adverse matrix conditions during the first 3 days of fermentation, with some strains keeping viability up to 6 days. An increase in the antioxidant activity was detected following fermentation with *L. casei* and *L. plantarum*. Almost all the strains increased the TPC values during fermentation, being yeasts more active than lactic acid bacteria with species- and strain-related differences. Aroma profile of the fermented clementine BPs was positively influenced by fermentation being enriched mainly in terpenes and alcohols. Overall, the selection of the microbial species and strains tailored for the specific features of clementine BPs is crucial for the development of

successful fermentation resulting in desired characteristics and enhanced functional properties.

Recent advances in 1H NMR wine analysis.

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Proton nuclear magnetic resonance (^{1}H NMR) shows great potential in wine authentication, quality control, assessment of climate and viticultural techniques, or monitoring production processes. It stands as a leading foodomic application of NMR. Multiple protocols exist differing in buffer used, buffer strengths and types, pH adjustments, freeze-drying or solid-phase extraction, each with their pros and cons. Challenges such as peak drift persist, with data extraction mostly relying on binning or peak alignment. No standardized acquisition method has emerged, though eightfold peak suppression is notable for its versatility. However, 1D NOESY pulse sequence with presaturation offers benefits by retaining ethanol signals, despite having lower signal-to-noise ratios for minor metabolites. Metabolomics data is processed using untargeted or semi-targeted approaches, along with unsupervised and supervised analytics. In the field, unique graphical outputs were established, which help to understand the main findings. Yet, many of the published studies are still pilot-scale with limited validation. Legal adoption of these methods for classifying wine varieties and origins remains distant. This presentation reviews recent advancements, discusses practical aspects, and suggests future directions for ^{1}H NMR in wine analysis.

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Microbial consortia for the development of new plant based fermented products.

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The growing interest in plant-based sustainable foods is fostering advancements in the creation of alternatives to animal-derived products. Fermentation emerges as a promising technology to enhance consumer acceptance of plant-based foods by offering sensory, nutritional, and safety improvements to raw materials used in manufacturing. Traditional food fermentations rely on diverse microbial consortia, including Lactic Acid Bacteria (LAB), Staphylococcus, yeasts, and filamentous moulds.

With a wealth of knowledge available on microorganisms and their industrial applications, we pose the question: can we reconstruct microbial consortia from established fermentations and apply them to plant-based substrates? This research aims to leverage the natural biodiversity preserved in microbial collections to construct microbial consortia specifically tailored for fermenting plant-based substrates. 50 strains of food origin from University of Parma Culture Collection (UPCC) and University of Bologna collection were genetically characterized using the Amplified Fragment Length Polymorphism (AFLP) technique.

The results reveal genotype-level differences among isolates from diverse food sources. Subsequent phenotypic screening of these strains, employing a metabolomic approach, unveiled their potential to utilize various substrates as energy sources. Integrating genotypic and phenotypic data provides valuable insights into the metabolic capabilities of industrially relevant strains, unveiling their potential application for biotransformation of plant-origin substrates.

Understanding the metabolic properties of individual strains facilitates the formulation of microbial consortia that can be applied in the development of innovative fermentation processes. This research contributes to the exploration of sustainable and plant-based alternatives through the strategic assembly of microbial communities with desirable characteristics for fermentation.

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Effects of probiotic Bacillus velezensis MT9 on tilapia fillets metabolomics profiles assessed by nuclear magnetic resonance spectroscopy.

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Probiotics have many benefits in aquaculture as they improve growth performance, feed use, immune defense against pathogens, disease resistance, stress tolerance capacity and water quality [1]. FishRISE is a multidisciplinary research project aimed at developing methodological and technological frameworks for aquaculture with a high index of environmental, social and economic sustainability. This study analyzed the muscle (fillet) of tilapia (*Oreochromis niloticus*) fish individuals subjected to controlled feeding with a probiotic (*Bacillus velezensis* MT9) over a period of 3 months to evaluate possible occurring nutraceutical benefits.
The investigation involved the combined use of ^{1}H NMR and multivariate statistical analysis (PCA, OPLS-DA) applied to the aqueous and lipid extracts [2] of fish muscle samples. Our metabolomics approach allowed observing differences related to both fish growth stage and used feed (± probiotic). Compared to controls, muscle samples extracts of probiotic fed fish

showed statistically significant increases in glycine, lactate, alanine, DUFA, PUFA, linoleic acid and EPA and lower concentrations of taurine, TMAO, creatine, histidine, IMP, 6P glucose and MUFA. Further studies are in progress to fully examining the beneficial changes in the metabolomic profiles of tilapias treated with probiotics.

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A machine learning approach for a straightforward inference of metabolites levels from microbial expression profiles in surface-ripened chees.

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Cheese is one of the most consumed dairy products in the world. The overall appeal of every cheese variety is related to its quality and flavors. These flavors result from the metabolic functions of the different microbial species participating in cheese fermentation and ripening, therefore, the gene expression profiles of these microbes can be used as a proxy to assess and monitor the flavor outcomes during cheese making. Here, we describe a machine learning approach to infer the amounts of six important classes of flavor compounds in an experimental surface-ripened cheese composed of nine microbial species based only on transcriptomics data. The predictive models were tested on an independent data set and the prediction accuracy ranged from 52 to 80 %. Our results provide a step towards characterizing cheese production aromatic outcomes from transcriptomic data of microorganism population involved in the process, helping to identify key factors and pathways.

New aptamer-based proteomic technologies: uncovering novel biomarkers of nutrition and health.

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The intricate interplay between nutrition and human health remains an enigmatic domain due to an insufficiency of comprehensive knowledge. This lack of understanding is largely attributed to the paucity of reliable biomarkers capable of enlightening the intricate relationship between nutrition and health outcomes. To address this crucial gap, novel proteomics technologies such as aptamer-based assays have emerged as

promising solutions. A proteomic platform (e.g. the SomaScan), exploiting the potential of aptamers, presents an innovative approach aimed to advance biomarker discovery and analysis in the context of nutrition and health.
The integration of such technology offers a new avenue for accelerating our comprehension of how dietary factors impact human health enabling the determination of over 11000 proteins related to the main biological pathways. By allowing the simultaneous and precise measurement of numerous proteins in small volumes of biological samples, this innovative proteomic technique holds the potential to unravel the complex network of interactions between the diet and individual metabolic and physiological responses, thus advancing our understanding of the intricate relationship between nutrition and human health. This advancement promises to significantly contribute to the optimization of dietary intervention studies and personalized nutrition strategies.

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Enrichment of pasta in antioxidant compounds: from raw material to cooked pasta.

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The importance of antioxidant compounds in foods and for the human body is well known and it is mainly due to their oxidative stability and functional properties (1). The aim of the study was to evaluate the antioxidant effect of used ingredients also to differentiate the gastronomic offer for consumers. In particular, pasta was enriched in antioxidant compounds (phenols and tocols) with the use of tomato and spinach powder in the recipe. The concentrations of antioxidant compounds were assessed in raw flours, and in crude and cooked pasta in order to evaluate the effect of the enrichment and the influence of the processes on the final products. Phenols and tocols from four different samples of pasta (pasta made with semolina, whole grain semolina, 70 % semolina and 30 % tomato powder, and 70 % semolina and 30 % spinach powder) and their relative raw flours were extracted (2,3) and analyzed with the Folin-Ciocalteu method (4), the DPPH assay (5), the HPLC-FLD and the HPLC-ESI-QqQ-MS (6). The whole wheat semolina showed a total phenol content and antioxidant activity higher than the semolina but also a greater sensitivity to degradation during production. Conversely, in the case of tocols, semolina was generally richer than whole wheat semolina and this trend was also found in the corresponding pasta samples. Although the enrichment with vegetable flours improved the total phenols content and the antioxidant activity, a strong decrease in these bioactive compounds was recorded in the final products due to the production process. Differently, the enrichment of semolina paste with vegetable flours determined a variation in tocols concentration, even if without significant differences in tomato-enriched pasta. In cooked pasta samples no differences were repoted in free phenols amount, while the bound phenolic acids strongly increased.

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COMBINR: Characterization of Multiomics Biomarkers in Lifelong Nutrition.

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The COMBINR project, part of Spoke 5 within the PE10 ONFOODS initiative, focuses on establishing a comprehensive set of Standard Operating Procedures (SOPs) for systematic multiomics data collection and analysis. Its main goal is to identify a methodological framework for pinpointing key aspects in multiomics studies, discerning distinctive -omics signatures as innovative biomarkers. SOPs have been meticulously crafted, from state of the art practices[1], for the collection and storage of clinical studies samples, microbiome analysis through next-generation sequencing, metabolome analysis via LC-MS/LC-HRMS and ^{1}H NMR spectroscopy, and tailored bioinformatics analyses for microbiome and

metabolomics data[2,3]. This comprehensive approach extends to machine learning and statistical methodologies for multiomics data integration, feature selection, prediction, classification, and biomarker discovery, fostering a holistic understanding of intricate biological processes.
The project delves into diet biomarkers in healthy donors for fecal microbiota transplantation (FMT) and women with breast cancer and osteoporosis, conducting a thorough investigation of diet-related -omic signatures in these patient groups for a deeper understanding of the relationships between diet, microbiota, and specific health conditions.

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Einkorn-based or semolina-based pasta: which is the best choice as source of bioactive compounds?

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The aim of this work was to analyze the chemical/nutritional composition of an einkorn-based pasta (pilot) and compare it to its counterpart benchmark. Pasta cooking conditions were standardized and the analysis were performed on raw and cooked pasta, as well as on the corresponding digested samples. Regarding the profile of the major lipid classes, a higher content of free fatty acids (FFA) and partial glycerides were found in einkorn pasta samples before and after cooking. This tendency was maintained even after cooking due to the chemical hydrolysis that occur during boiling. As for triglycerides, however, higher values were found in benchmark samples compared with pilot ones formulated with einkorn flour. After *in vitro* digestion, the most abundant lipid class turned out to be FFA, while still maintaining the same lipid class profile found in the samples before digestion. Regarding the fatty acid (FA) composition, einkorn-based pilot and benchmark raw pasta had similar levels of saturated (SFA ≅ 17-19% of total fatty acids), monounsaturated (MUFA ≅ 19-20%), and polyunsaturated fatty acids (PUFA ≅ 58-63%). However, cooking led to an increase in PUFA and a decrease in MUFA in both types of pasta, while SFA concentration was not affected. Furthermore, essential fatty acids followed the same pattern as PUFA. The PUFA/SFA ratio (≅ 3.2) resulted to be similar in both analyzed products, while the *n*-6/*n*-3 ratio was higher in pilot pasta (18 *vs.* 14). On the other hand, after *in vitro* digestion, there was a decrease in the content of PUFA and EFA and an increase in MUFA; however, the *n*-6/*n*-3 ratio remained almost constant, being always higher in the pilot pasta. In the case of the sterol content, it was 2 times higher in the einkorn-based pilot pasta than in the benchmark product. Moreover, cooking had a major impact in wholegrain semolina products, leading to a 42% loss in total sterols, compared to the pilot pasta (29%). Regarding the phenol composition, more insoluble phenols were found than soluble ones in both types of pasta samples before and after cooking, as well as after their *in vitro* digestion. Finally, the tocopherol content in the einkorn-based pilot samples was 2-times higher than that of the benchmark pasta. In conclusion, these results allow us to state that, in comparison with the benchmark, the pilot pasta had a higher content of bioactive compounds, which remained in the product at higher concentrations after cooking, as well as after *in vitro* digestion. These bioactive compounds could thus potentially exert their beneficial role on human health, representing a valorization trait for this food supply chain and the pilot product commercialization.

The present work was developed within the activities planned by the EU-funded project GourMED (Prima 2-2020) "Governance of Food Supply Chain to Equilibrate Price and Profits of High Quality and Safe Mediterranean Foods".

Vinaccia Goldmine Unveiled: Unleashing Marche Wine Grape Pomace's Potential in Gastric Cancer Prevention

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Gastric cancer remains a significant global health concern. The ongoing quest for effective chemopreventive agents has yielded promising results, underscoring the potential of various natural compounds in cancer prevention. [1] Grape pomace, a winemaking byproduct rich in polyphenols, holds promise as an antitumor agent. Repurposing vinaccia aligns with circular economy principles, benefiting both economics and health. Utilizing a Soxhlet extraction technique, a phytochemical complex was obtained, subsequently subjected to both chemical analysis and biological testing to assess its effects on gastric carcinoma cell lines AGS, KATO, and SNU-1 cell lines through Scanning (Fig. 1 A, B, E, F, H, I) and Transmission Electron Microscopy (Fig. 1 C, D, G, L) Chemical analysis revealed the presence of phenolic acids, flavonoids, and smaller amounts of stilbenes. (Fig.1) Grape pomace doses (1.25μ and 2.5μ) induce necrotic, apoptotic changes, and bleb formation in AGS cells, visible under Scanning Electron Microscope (SEM) (A, B). Transmission Electron Microscopy (TEM) reveals cytoplasmic vacuolization, mitochondrial damage, and micronuclei in AGS cells (C, D). KATO cells under similar treatments show shape changes and surface blebs (SEM) (E, F), with mitochondrial damage and autophagic vacuoles (TEM) (G). SNU-1 cells exhibit more

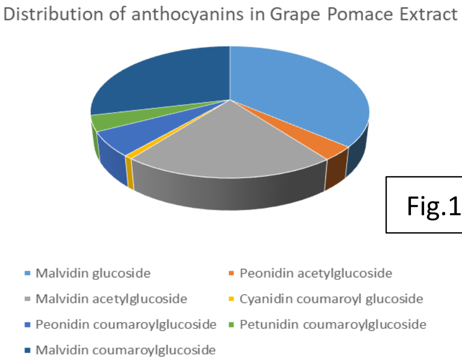

Fig.1

resistance, with occasional blebs (H, I) and mitochondrial damage (L).

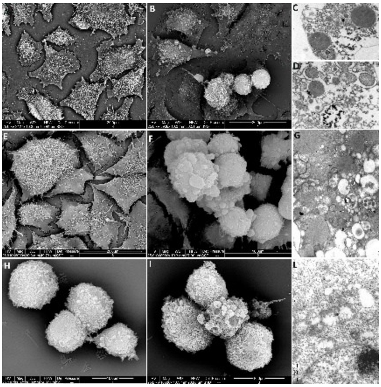

Fig.2

(Fig.2) This study suggests grape pomace extract's potential as a nutraceutical, enhancing health and sustainability.

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Lipid bioaccessibility in four different supplements of omega-3 fatty acids.

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The steady growth of the global population and the need to adopt a more sustainable diet is driving the research to identify new sources of omega-3 (n-3) long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), key nutrients for the prevention of several cardiovascular diseases. *Calanus finmarchicus* represents a sustainable source of n-3 LC-PUFAs, mostly in the form of waxes. Since it is still unclear whether the human body can digest waxes, this research aimed to evaluate the lipid bioaccessibility in four commercial supplements based on encapsulated calanus oil (2 brands, CO-1 and C-O2), krill oil (KO) and fish oil (FO). The supplements were digested with and without capsules using the INFOGEST method (1). The lipids were extracted from non-digested and digested supplements using the Bligh&Dyer method (2), and the fatty acids were methylesterified according to Stoffel (3) and analysed by GC-FID as methyl-esters (FAMEs).

The lipid content of not digested supplements was similar. The amount of lipids released from the matrix after digestion was independent of the presence of the capsule in all samples except KO, in which the capsule strongly inhibited the release. In capsulated supplements, lipid bioaccessibility was in the order FO>CO>>KO. Using the Stoffel method, it was not possible to derivatize fatty acids esterified in waxes, and further experiments are ongoing to evaluate FAMEs in CO. The amount of FAMEs was similar in not digested FO and KO supplements, while the content of FAMES in the bioaccessible fraction of digested FO was higher than FO. No significant differences in the fatty acid profile were detected comparing the same oil before and after digestion, regardless the presence of the capsules. KO had a higher content than FO of alpha-linolenic (C18:3 n-3), stearidonic (C18:4 n-3) and eicosapentaenoic acid (C20:5 n-3), while the longer and more unsaturated fatty acids, docosapentaenoic (C22:5 n-3) and docosahexaenoic (C22:6 n-3) acids were more concentrated in FO than KO. Our study revealed significant differences not only in composition but also in digestibility of omega-3 fatty acid supplements. Studies are underway to evaluate fatty acid uptake in Caco-2 cells.

This study was supported by the EU Commission, BLUEBIO COFUND-H2020 project "BIOZOOSTAIN" (ID 817992) and by the Italian MUR, project "ON Foods" (ID PE00000003).

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Nutrition declaration. Is it time to move beyond chemical composition?

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Among macronutrients, lipids have the highest energy supply. According to the DRI, it is recommended that they contribute a maximum of 35% of daily energy intake. In particular, saturated fats should not exceed 10% of total energy. Consumption of high-fat diets is associated with an increased risk of various diseases. To promote awareness about the importance of restricting high-fat foods, both total and saturated lipids are reported on the “nutrition facts” label and considered in the nutrient score calculation.

Nevertheless, the nutrient score and nutritional label are based on the chemical composition of the food and do not consider bioaccessibility, that is the percentage of the nutrient that is released from the food matrix during digestion and which becomes available for absorption. Since the food matrix strongly influences bioaccessibility, different foods may have similar lipid content, the absorption of which may be different.

In this study, we assessed the bioaccessibility of lipids in four commercial, ready-to-eat foods: biscuits, nuts, canned pickled mackerel and cream cheese. Foods were digested in vitro according to the INFOGEST protocol (1) and lipids were extracted from the soluble, bioaccessible fraction and from the pellet using two different methods (2, 3). The lipids were weighed, methyl esterified and the fatty acid methyl esters quantified by gas chromatography.

Regardless of the extraction and quantification methods employed, the proportion of lipids released from the food matrix during in vitro digestion, and hence potentially absorbed, was different in the four foods. This highlights that the fat concentration may not reflect the physiological effects of the food, including its energy content, and the bioaccessibility of lipids should be carefully considered.

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Resilience Plan (NRRP), CUP D93C22000890001, project "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods" (Project code PE00000003).

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Bacterial cellulose production by selected acetic acid bacteria on different Agrifood by-products.

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Bacterial cellulose (BC) is produced by certain acetic acid bacteria (AAB) and represents a valuable biopolymer due to its interesting mechanical and technological properties. However, the production cost represents the main limitation to the industrial production of this biomaterial. In this scenario, Agrifood by-products represent a cheaper and naturally rich sources of nutrients which are suitable for microbial fermentation and thus, for BC production. In this study, the growth kinetics and BC production of *Novacetimonas hansenii* **6DB**, *Komagataeibacter rhaeticus* **LM2, LM4** and **SM1** and *Komagataeibacter xylinus* **DSM 2004** were investigated using beet molasses, brewer’s spent grains and yeast and cheese whey as growth media.

The results revealed that BC productivity was affected by the AAB strain and agrifood by-product considered. Under optimized conditions, the highest BC yields, after 10 days of incubation at 28°C, were achieved by the strains **DSM 2004** (2.878 g/L) and **LM4** (1.698 g/L) using beet molasse as growth medium. Lower levels of BC were produced in brewer’s spent grains alone whereas when combining it with brewer’s spent yeast and phosphate buffer all the AAB showed a BC dry weight higher than 1.1 g/L. The pH value of beet molasses and cheese whey media slightly decreased whereas that of the brewer’s spent grains medium was found to significantly decreased during the incubation at 28°C when the buffer was not added to the medium. It was also observed that cellulose production gradually increased with the increase of incubation time, but the rate of BC formation remained almost constant after 7 days. Beet molasse medium proved to be a more suitable substrate for BC production in comparison with brewer’s spent grains and cheese whey. Overall, this study demonstrated the potential of producing BC from wastes. However, a good understanding of the factors affecting BC production and the identification of the optimum conditions for a low-cost-effective BC production could significantly incentivize the expansion and the potential applications of this biomaterial in various industries and especially in the food industry.

Effect of High Pressure Homogenization on Rheological and Functional Properties of Chickpea Flour and Its Two Main Components: Starch and Protein

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In order to formulate innovative food products with chickpeas, this study aims to observe the behavior of chickpeas and their two main components, chickpea starch and protein, processed by high pressure homogenization (HPH). To investigate the functional and rheological properties of the components, starch and protein were extracted from whole chickpea flour. The extracted components were treated at different pressures (30, 60, 90, and 120 MPa). To determine the effects of the treatments, the functional and rheological properties of the components were evaluated. Overall, the chickpea flour showed some increase in water, oil holding capacity and water adsorption index, due to the HPH treatment. This could be because HPH treatment affects the structure of the protein and enhances the interactions between protein and water. However, chickpea flour showed some decrease in its water solubility index and protein solubility. These changes could be due to the fact that protein unfolding was triggered by increased protein aggregation, due to the increase in pressure.

After the treatments, different behaviors were attributed to changes in the starch and protein fractions of the flour, confirming the potential of HPH to alter the functional properties of the components.

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Assessment of the quality of sustainable sources of omega-3 supplements as approached by the Foodomics perspective.

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In recent years, many efforts have been made to find new sources of long-chain omega-3 polyunsaturated fatty acids (ω-3 LC-PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), hopefully with high bioavailability to be considered of high nutritional value. However, the main problem is that the molecules of interest are present in complex mixtures that require precise definition if they are to be used for human nutrition. The description is rather easy for what represents the expected main fractions, while there is great uncertainty on the chemical and functional nature of what can be defined as impurities, which are sometimes equally important in defining the general quality of the product to be administered.

As part of the European BIOZOOSTAIN project (*), a new "green" source of ω-3 LC-PUFA has been identified in the fractions extracted from fishing by-products. The aim of the present study was to define, through a Foodomics approach, an analytical platform for the characterization of the different sources of ω-3 LC-PUFA, coming from the upcycling of by-products of marine origin, otherwise considered waste.

Marine oils of different origins (fish, krill, Calanus) were analyzed by high resolution 1H-NMR spectroscopy to identify and quantify the molecules of interest. Through unsupervised multivariate analysis (principal component analysis), the portions of the spectra suitable for carrying out an overall evaluation of mixtures of different origins were selected.

In fact, quantitative analysis is often hindered by the overlap of the signals belonging to the molecules of interest with other underlying signals of impurities which lead to an overestimation. To exclude possible interferences from the integration, a dedicated algorithm has been developed for selecting the points of the spectrum to be subjected to integration for quantitative analysis. The steps that follow one another in the developed procedure include: i) construction of a correlation matrix of the spectral buckets; ii) selection of the most autocorrelated spectral regions, iii) application of PCA on the selected spectral intervals, iv) analysis of the loadings to identify the best set of spectral points, i.e. the "cleanest" from interference, on which to measure the integral to establish the concentration of the corresponding molecule. The results obtained are comparable with those measured by gas chromatography, with the advantage that, through NMR spectroscopy, it is possible to identify the quantity of the molecules of interest, without setting up the separation conditions for each kind of mixture, as required by the GC. Furthermore, since the standard is not necessary for NMR, to obtain the response factor between quantity and area, it is possible to "clean" the spectrum even after the analysis.

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Acute and long-term effect of a Mediterranean dietary pattern providing higher or lower carbohydrate intake on physical performance and biochemical markers in non-professional strength trained athletes.

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One of the most well-known and studied dietary patterns worldwide is the Mediterranean diet (MD). However, its importance and advantages in sport nutrition contexts receives very little attention. Consequently, the aim of the present study has been to evaluate the effect of MD providing different amount of carbohydrates on different parameters such as strength, physical performance, body composition and blood markers by conducting a crossover randomized trial in a group of non-professional sport athletes who practice a strength activity.

This study enrolled volunteers that completed an 8-weeks dietary intervention in a randomized, crossover design. The interventions included two different dietary intervention: a MD High in Carbohydrates (HCMD), characterized by 55-60% of daily energy derived from carbohydrates, or a-MD moderate in Carbohydrates (MCMD), with 40-45% of daily energy provided by carbohydrates.

Thirteen subjects (9 males and 4 females) completed both interventions. No statistically significant time for treatment interactions were recorded for all variables with the exception of biceps strength (repeated measure ANOVA). Focusing on intragroup data, no differences in biceps strength in the HCMD group (P=0.490) was found, whereas the MCMD group showed a significant decrease overtime (P=0.002). No significant changes were found between baseline and after HCMD or

MCMD in squat jump, countermovement jump, biceps, handgrip, and maximum voluntary contraction. The hematological profile was not affected by the different diets, while a significant post-intervention decrease in arm circumference was observed in the MCMD group ($P < 0.05$).
This study shed in light that reducing the amount of total carbohydrates in the context of mediterranean diet did not significantly improve the parameters of performance, body composition and strength in non-professional strength trained athletes.

Exploring the effects of Bergamot (citrus bergamia) juice on viability of C2C12 myoblast cell line.

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The bergamot (Citrus bergamia) juice extract (BJE) has shown protective effects against muscle wasting through its anti-inflammatory and antioxidant effects due to two major polyphenols: Bergamjuicin glucoside and Neoesperidin-glucoside. The aim of this work was to elucidate the cytotoxicity of a polyphenol rich extract obtained from bergamot juice on skeletal muscle cells (myoblasts C2C12) in an experimental model.

The C2C12 cell line, an immortalized mouse myoblast cell line, was purchased from the American Type Culture Collection (Manassas, VA, USA)

Cells were treated with increasing concentrations (1, 10, 30, 60, 120, 240 mg/mL) of a polyphenol rich BJE for 24 hours. The cytotoxic effect of (BJE) on C2C12 myoblasts was determined by the MTT assay. Inflammation and oxidation were induced in C2C12 with LPS- and H_2O_2 treatment. Cells were pre-incubated with BJE. At last, viability was measured by MTT assay.

Results have shown that at concentrations from 30 to 240 mg/mL increases cell metabolism from 30 to 40%. Besides the anti-inflammatory and antioxidant activity of BJE in an inflamed model of C2C12 have been explored.

This study revealed no reduction in myoblasts C2C12 cell viability in response to BJE. Surprisingly, BJE caused a dose-dependent increase in viability. Defining the protective effects of BJE against different stressors could lead to personalized diet therapies in subjects with muscle wasting.

Metabolic Profiling of Green Coffee Beans from Diverse Geographical Origins.

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The investigation of plant metabolic responses to environmental factors can be extremely useful for a better understanding of the relationship between agricultural practices, food quality and health. This study is focused on identifying the metabolic profiles of green coffee beans (*Coffea arabica* L.) from different geographical origins worldwide. Supplied by *illycaffè* S.p.A. in Trieste, the green coffee samples from Brazil, Guatemala, Honduras, Ethiopia, Rwanda, and India were analyzed to evaluate the presence and concentrations of biogenic amines and various bioactive compounds, such as alkaloids and phenolic compounds.

High-performance liquid chromatography with different detection methods (Fluorescence Detection and Diode Array Detection) was employed to quantify biogenic amines, caffeine, ferulic acid, and chlorogenic acids (5-Caffeoylquinic acid, 3-Caffeoylquinic acid, and 4-Caffeoylquinic acid).

By exploring whether the characterization of bioactive compounds in green coffee beans can serve as a molecular marker for determining their geographical origin, this research can contribute to the discussion on the complex biochemical processes within coffee beans and their potential implications for food quality, while encouraging consideration of innovative methodologies for scientific investigation into nutrition and health.

This study was carried out within the Agritech National Research Center and received funding from the European Union - NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Protease inhibitors in soy-based analogues.

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Plant-based alternatives to dairy and meat products are becoming widespread due to increased consumer awareness of the environmental impact and possible health problems associated with excessive consumption of animal products [1]. However, in addition to the numerous advantages that the use of plant sources can bring, some possible drawbacks must be considered. Among these, the possible presence of anti-nutritional factors such as protease inhibitors. These compounds, if not completely removed or inactivated, can compromise the digestion of proteins, leading to a reduction in their biological value. One of the main alternatives to animal proteins in human nutrition is soy, a protein-rich legume. However, soy is also known to be a source of antinutritional factors such as the Kunitz and Bowman Birk inhibitors, which inhibit trypsin and both trypsin and chymotrypsin, respectively. A UHPLC/ESI-MS/MS method has been developed to accurately quantify these two molecules in soy-based foods [2], both raw and processed, which will allow the actual content of these anti-nutritional factors in soy-based analogues of dairy and meat products to be assessed.

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Low sodium cheeses lipidic characterization, a research to better understand the kinetics of cheese aging.

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Cheese is a product created to preserve the nutrients of milk. It is obtained through acid or rennet coagulation of whole, partially, or fully skimmed milk, or cream, using cultures or kitchen salt, it is a source of proteins, fats, minerals, and vitamins, and it serves as a historical, traditional, and fundamental food in many cultures worldwide. In most traditional and industrial cheese production systems, the cheese forms undergo a salting process, which normally requires the use of sodium chloride [1]. The accumulation and overconsumption of sodium are associated with conditions such as arterial stiffening, high blood pressure, kidney stones, and energy imbalances [2]. This study aims to investigate the effect of salting on the kinetics of lipolysis and proteolysis in cheeses, produced in pilot implants, characterized by intermediate aging, to better understand if and how the use of lower sodium concentrations affects the aging kinetics. The study was performed by combining standard chemical and instrumental analyses for lipid characterization and oxidation, namely volatile compounds analysis and diacylglycerols analysis, with td-NMR analysis on cheeses produced in pilot implants at DISTAL fields in Bologna. The cheeses were all made with fresh milk purchased from local farmers, the starter culture consisted of *Latilactobacillus* (LYOFAST 102) and *Streptococcus thermophilus* (LYOFAST 022) and said products were placed in a solution of water and salt at 24° °C ± 1°C for different periods of time, ranging from 1 hour to 4 hours. Subsequently all the cheeses were stored for different periods of times as indicated in table 1. Results, particularly regarding the combined effects of sodium chloride, on both the lipolytic and proteolytic products, highlighted how a reduced amount of salt produced similar performances to standard use, describing also a possible increased lipolytic activity in cheeses produced with standardized quantities. The next step in the research will be the creation of a Panel protocol and the put in place of microbiological challenge test to confirm on both the sensorial, and safety aspect the viability of low sodium protocols for cheese production.

Days of aging	0	15	30	60
0 hours of salting	4a	/	/	/
1 hour of salting	/	1a	2a	5a
2 hours of salting	/	1b	3b	4b
4 hours of salting	/	2b	3a	5b

Table 1: production sampling of the analyzed cheeses

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Pulse electric fields and ultrasound technologies for extracting phenolic compounds from avocado by-products.

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Avocado (*Persea americana* Mill) is a tropical fruit wide consumed which production was 8.69 million metric tons of avocado in 2021. After food processing, around 40% of the whole fruit is discarded as by-products (peels and seeds). These by-products are a source of phenolic compounds, specially procyanidins. Thus, this study focuses on optimizing the conditions for pulsed electric field (PEF) technology as a pretreatment to obtain extracts rich in phenolic compounds from the avocado peel and seed. Two Box-Behnken designs of 15 experiments with three levels (-1, 0, 1) were carried out. The independent factors in the case of avocado peel were frequency (1, 50, 100 Hz), voltage (1500, 2250, 3000 V) and number of pulses (100, 150, 200), and for the avocado seed frequency (50, 100, 150 Hz), total operating time (1, 5, 10 s) and electric field (0.6, 0.7, 0.8 kV/cm), established based on preliminary experiments. The dependent variable in both models was the procyanidins content in the extracts obtained by ultrasound technology via sonotrode and analyzed by HPLC-FLD. The models were confirmed and validated by ANOVA. The optimized parameters chosen for the avocado peel model included a frequency of 50 Hz, 2625 V, and 175 pulses, resulting in a desirability value of 0.9468. This configuration predicted a procyanidin content of 9845.41 ± 222.60 µg CE/g d.w. On the other hand, for the avocado seed model, the selected conditions involved a frequency of 100 Hz, a field strength of 0.75 kV/cm, and a total operating time of 5.5 s, yielding a

desirability value of 1 and a predicted procyanidin content of 20955.06 ± 452.35 µg/g d.w. They were achieved significant ($p < 0.05$) increases of 9.5 and 31.5% in the procyanidin content in avocado seed and peel, respectively, compared to only using ultrasound extraction. The combination of optimized PEF pre-treatment with ultrasound extraction has to obtain avocado by-products enriched extracts in procyanidins that could be used for food, nutraceutical, and cosmeceutical scopes.

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It takes two to tango: Let's use mechanical stimulation to enhance the expression of differentiation-related genes in an enterocyte co-culture model to study the interplay between food and the intestinal epithelium.

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The development of an accurate *in vitro* intestinal model requires integrating a simulated digestive system with cell cultures that mimic the human intestinal epithelium. Caco-2 cells are often used to evaluate permeability, but accurately reproducing the intestinal environment *in vivo* remains challenging [1,2]. To overcome a major limitation of Caco-2 cells –their lack of mucus secretion– we propose a model co-culturing Caco-2 with mucus-producing HT29-MTX goblet cells seeded in Transwell at a 9:1 ratio. This co-culture better reflects the diversity of intestinal cells *in vivo* and creates a more representative enterocyte model. However, it is still difficult to improve the structural organization, differentiation and mucus secretion of the cultured intestinal epithelium [3]. The aim of this study was to improve this co-culture model by focusing on the expression profile of genes related to polarization (*CDH1* and *TJP1*), differentiation (*ALPI*, *DPP4*, and *SI*) and mucus secretion (*MUC5AA* and *MUC2*) from confluence (day 0) to different time points (day 7 and day 14) until full differentiation (day 21) under static and dynamic conditions. To monitor the integrity of the cell barrier, transepithelial electrical resistance (TEER) was also measured at every other day after medium replacement. We had evidence that growth of the enterocyte co-culture on an orbital shaker (Celltron, INFORS HT; MD, USA) led to an improved cell organization and a more goblet-like phenotype compared to static culture, thus showing greater similarity to the intestinal epithelium [4,5]. As a case study, a model food (skimmed milk powder, SMP) was *in vitro* digested (Mulet-Cabero *et al.* 2020 [6] with some modifications to ensure the biocompatibility [7]) and added to the enterocyte co-culture model at different concentrations (1:3 – 1:20 dilution with cell culture medium) for 3 h on day 21 to investigate the effect of the digestate on cytotoxicity. Using the Alamar blue assay, none of the treatments tested were found to be detrimental. Confocal imaging was used to assess cell morphology of cells treated with digesta by staining cells with MitoTracker vital dyes, fluorescent lectins and nuclear labelling. Ongoing experiments are testing the *in vitro* integrated system by adding titanium dioxide nanoparticles to SMP at the maximum concentration allowed by the FDA for food use [8].

This study was funded by the ALMAIDEA 2022 program of the University of Bologna under the project 'GREENER - How the complex physical-chemical and physiological events present in the human GastRointEstinal tract can intEract with NanomatERials potentially present in food: Journey from inorganic to biological using advanced in vitro models'.

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Edible insects metabolite characterization by using a multimethodological approach: the case study of Acheta domesticus

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The research of new and innovative food sources represents an ever-growing need, considering the expected large population increase and the necessity of reducing the ecological problems related with intensive food production [1]. In this context, in the last years, the European Union has started to consider edible insects as a potential food source capable to satisfy both sustainability and nutritional demands. The recent introduction of *Acheta domesticus* (house cricket) in the official European list of Novel Food [2], has requiring an improvement of its chemical profile knowledge. In the present work, for the first time, a spray-dried *A. domesticus* powder was investigated by means of a multimethodological approach based on NMR, FT-ICR MS, and GC-MS methodologies. Several classes of compounds namely amino acids, organic acids, fatty acids, terpenes, and other metabolites were identified and quantified. Moreover, the proposed analytical protocol allowed to identify and quantify compounds not previously reported in cricket. In particular, methyl-branched hydrocarbons, previously identified in other insects, together with other compounds such as citrulline, formate, *γ*-terpinene, *o*-cymene, *α*-thujene, *β*-thujene, and 4-carene were detected. The improved knowledge of the chemical profile of this Novel Food opens new horizons both for the use of cricket as food ingredient and for the use of its extracts for the production of new formulations.

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Enhancing Food Quality Analysis: Deep Neural Network Inversion of NMRD Profiles with Quadrupolar Dips.

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Fast Field Cycling (FFC) Nuclear Magnetic Resonance Dispersion (NMRD) is an evolving technique giving detailed insights into the molecular dynamics of food products, useful in quality control, shelf-life estimation, and the verification of denomination of origin. The sensitivity of NMRD profiles to various factors such as dehydration, oxidation, spoilage, and the presence of additives provides a powerful means to detect adulteration and ensure product authenticity. However, the interpretation of NMRD profiles, particularly in the presence of quadrupolar dips, can be challenging due to their complex nature [1]. Recent advancements in artificial intelligence offer promising solutions to this challenge. Deep Neural Networks (DNNs), in particular, have proven good potential in inverting NMRD profiles effectively, even with the complexity introduced by quadrupolar interactions. This work presents preliminary results from employing DNNs for the inversion of NMRD profiles. The findings suggest that DNNs could become an integral component of NMRD profile analysis, offering a robust method for detecting fraudulent practices in the food industry.

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Spotlight on a 50-year evolutionary history of *Lactobacillus helveticus* from natural whey starters of Parmigiano Reggiano.

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Lactobacillus helveticus is a homofermentative LAB species associated to natural whey starters (NWS) for traditional cheese-making such as Parmigiano Reggiano (PR), that shows a great phenotypic and genotypic biodiversity, also among strains of the same ecosystem. With the purpose to provide a deeper insight in the variability of *L. helveticus* strains, the project Helv4Dairy aims to study the changes that might have undergone in the last 50 years in terms of bacterial adaptation to the specific ecological niche represented by NWS. In this perspective, -omics techniques can shed a light on the *L. helveticus* adaptation and evolution in NWS due to the environmental selective pressure associated with technological advancement in dairy practices or to abiotic changes (antibiotics exposure). Indeed, along the years, PR manufacturing could have easily brought to the loss of “old” strains, substituted by new adapted ones with different traits.

The availability of an extensive *L. helveticus* collection built in 1970 at DISTAL can provide a one-of-its-kind picture of *L. helveticus* features in NWS of 50 years ago. In fact, after their isolation and identification according to the methodology and taxonomy of 50 years ago, the strains were lyophilized and never revitalized before. More than 20 strains have been revitalized, genetically identified and studied in comparison with some strains isolated recently from NWS in dairies from the same geographical area and considered as representative of the current biodiversity of this species in this environment. Preliminary data obtained on the LH isolates indicated a discriminant genetic diversity between strains isolated in 1970 and strains isolated in 2021. Besides, the strains from the 1970 collection showing a reduced antibiotic resistance (AMR), could represent a biotechnological strategy for mitigation of AMR genes spread in the dairy sector, promoting the sustainable use of microbial biodiversity in food ecosystems. In this perspective, a deeper insight in the genotypic and phenotypic features at different evolutionary conditions is crucial for the strain technological exploitation and for the selection of autochthonous starter cultures.

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AGRILOOP EU project: high value products from agricultural residues through sustainable chains.

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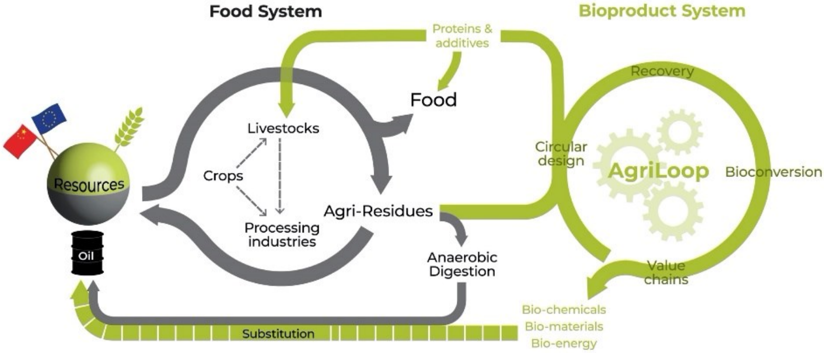
Agricultural-food residues are an underexploited resource for the European Union and China. Making better use of these will bring significant economic, environmental and societal benefits.

AgriLoop (https://www.agriloop-project.eu) is a major European Union, UK Research and Innovation and Chinese-funded project finding new ways to convert agricultural-food residues into high-value, eco-friendly products, such as food and feed ingredients and bio-based materials.



In relation to the food chain Agriloop will: 1) strengthen the relationship between China and the European Union, by working together on common tasks and objectives; 2) convert tomato, soybean, peanut, apple, potato, brewery grains, oil, grapes agro-industrial food residues into added-value ingredients; 3) develop new products and processes for the food, health and agricultural sectors; 4) apply a 'safe-and-sustainable-by-design' method that avoids or minimises harmful impacts. These methods in a cascading biorefinery approach include: green extraction, microbial conversion and material compounding and extrusion; 5) demonstrate innovative and sustainable value chains; 6) achieve several environmental, societal and economic impacts.

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Meat genomics comes of age: applications over the whole value chains, from animal breeding to meat authentication.

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High-throughput sequencing and genotyping technologies have been applied in meat animals for different purposes and from different perspectives. Here, we present different applications of genomic technologies for the sustainable development of meat animal value chains. We have applied genome-wide association studies (GWAS) and candidate gene analyses carried out within breeds or populations to identify genomic regions and markers associated with meat quality and production traits of the animals and novel molecular phenotypes directly or indirectly involved in muscle mass and fat deposition, feed efficiency, welfare of the animals and disease resistance. We have combined GWAS with population genomic analyses across breeds, including autochthonous animal genetic resources, to identify signatures of selection that can be used to identify genomic regions explaining the variability of relevant traits for

meat production and adaptation to peculiar production systems in cattle, pigs, and rabbits. We have then applied whole genome sequencing to identify new causative or putative causative mutations affecting the targeted traits in all investigated meat species. We have also applied genomics for the development and implementation of authentication and traceability systems of meat products, including the use of single DNA markers, multiple single nucleotide polymorphism panels, and targeted and untargeted whole DNA sequencing of DNA extracted from complex or simple meat-based matrices. Authentication DNA-based approaches have made it possible to strengthen the link between breed-branded meat products and the conservation of animal genetic resources, including autochthonous breeds, and to enlarge the application and sensitivity of the assays that can even easily recover information from the single animals from which the products have been obtained.

The Mutual Interactions of Silymarin and Colon Microbiota

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Dietary supplements, such as silymarin (a milk thistle extract), interact with the gut microbiome, influencing their effects. This study investigates the interaction between silymarin and the human gut microbiome across different age groups. The study involved *in vitro* fermentations with human stool of 20 human participants, belonging to groups: younger (<45 years) and older (>70 years). Advanced methods such as 16S rRNA sequencing, ^{1}H NMR, and LC-MS were used to analyse the start and endpoint of fermentation. Silymarin was transformed into 20 microbial catabolites. The most prevalent catabolites were demethylated silybins. Older participants had 2.5 times more of a specific catabolite (m/z 399.105, retention time 10.4), which positively correlated with *Oscillibacter* and *Anaerotruncus* spp. Silymarin had an impact on gut metabolite profiles, resulting in a decrease in the production of short chain fatty acids such as acetate (by 2.32%) and isobutyrate (by 8.16%), and a decrease in glucose utilization by 10.2%. These findings highlight the need to consider age in understanding the role of polyphenols for personalized nutrition across age groups.

Analysis of virgin olive oils volatilome: quality grade evaluation and study of modifications during storage

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Virgin olive oil (VOO) is a cornerstone of the Mediterranean diet, recognized for its unique aroma and its beneficial properties. The positive and negative sensory characteristics of VOOs are due to the presence of volatile organic compounds (VOCs). However, these attributes and their perceived intensity can change over time, due to factors such as oxidation which can be accelerated under inadequate storage conditions [1]. VOCs can be used to monitor changes in VOO characteristics selecting specific markers linked to defects (e.g. rancid) and molecules responsible for fruity or other green notes. For these reasons this study aims to profile the VOOs volatilome, tracking the evolution of selected volatile markers over a storage of several months. The samples were sensory classified according to the application of the official method, known as Panel test [2] and analysed with a semi-targeted approach by headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS), as support to the sensory assessment through the application of PLS-DA chemometric models.

Furthermore, VOCs were analysed by a targeted validated method using solid phase microextraction (SPME) in combination with gas chromatography (GC) coupled to mass spectrometry (MS). An integrated approach of sensory and metabolomic analysis can allow a more complete assessment of the modifications that occur in VOO during storage, monitoring potential changes in lipid oxidation markers and molecules responsible for positive and negative sensory attributes.

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Advance in YOUAREIN (YOUng AgRifood European INnovators) project: Syllabus developed!

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The YOUAREIN Project, co-funded by the European Union, is an ambitious initiative driven by the pressing need for innovation and sustainability in the food industry. With the increasing global focus on sustainable practices and the demand for innovative solutions, the project aims to empower and equip aspiring food entrepreneurs with the necessary skills and knowledge to thrive in this dynamic sector. Our project combines innovation, sustainability, and entrepreneurial education to create a powerful platform for aspiring leaders in the food sector.

The Syllabus of the course has been developed and it is structured into 4 stages: Ideation, Prototype, Business Plan and Scale Up. Each stage includes different modules which are then divided into some specific topics in order to facilitate the choice of lessons to follow by course participants. The preparation of the materials, which include a series of video lessons, interviews with entrepreneurs in the sector to be used as models and to study their business cases, is ongoing. The course will be available in September 2024, and it is addressed to undergraduates, graduates and PhD students, academic teachers and tutors, entrepreneurs in the food sector, food industries representatives, policymakers, as well as consumers interested in sustainable food production.

Chemical-physical and nutritional characterization of Yacon Juice Enriched organic strawberries.

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Health and sustainability trends have triggered consumers into choosing food products that are as natural as possible such as organic ones, having at the same time improved nutritional and functional properties. The fortification of organic fruit products with juices or solutions containing functional ingredients could be achieved through the application of vacuum impregnation (VI). Yacon juice is rich in fructooligosaccharides, in particular inulin, which exerts the prebiotic activity. Moreover, it has a high antioxidant activity due to the presence of polyphenols and vitamin C. The aim of this study was to obtain naturally fortified organic strawberries by their enrichment with Yacon juice. In the present work, organic strawberries cut in half were impregnated with organic Yacon juice by VI technique (200 mbar, 24 min). The impact of Yacon enrichment was investigated on quality characteristics of strawberries (colour, texture, weight gain), and nutritional characteristics such as Total Polyphenols, Vitamin C, Anthocyanins, Proanthocyanidins, Ellagic acid and Ellagitannins content, and antioxidant activity. The analyses were performed on the samples during refrigerated storage at 4°C. The results showed that a satisfactory impregnation of strawberries (of about 5%) was achieved along with the modification of the antioxidant compounds content and activity. Colour of strawberries were influenced by the impregnation with Yacon, turning to the dark red. During storage, impregnated samples showed a good stability of the bioactive compounds analyzed.

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Cold atmospheric plasma treatments for the decontamination of sliced carrots.

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In recent years, the request for safer and more sustainable food decontamination methods has led to several alternatives including the use of natural antimicrobials or innovative physical treatments. Among the latter, cold atmospheric pressure plasma is a promising alternative to traditional chemical sanitizers in food safety. This study represents a step forward in our understanding of cold plasma's potential applications, specifically focusing on its effectiveness in reducing contamination by some pathogens on sliced carrots. In pursuit of this goal, challenge tests were set up by inoculating carrot slices with a cocktail of two strains of *Escherichia coli* (~5 Log CFU/ml). Subsequently, the contaminated carrot slices were exposed to various treatments: i) gaseous cold plasma under NO_x or O_3 regimes; ii) washing with plasma activated water (PAW) in comparison to chlorinated water (100 ppm free chlorine). Specifically, treatments were carried out for 2, 5, 10, 20, and 30 minutes. Overall, PAW was poorly effective against *Escherichia coli* regardless the washing time, while the reference sanitizer resulted in aprrox. 2 Log reduction after a 2-min washing. However, no improvement was observed by prolonging washing up to 30 min. When direct cold atmospheric plasma treatments were performed, the NO_x regime showed higher performances compared to the O_3 one, with average inactivation values of 1.5 Log CFU/g. While further trials are necessary to evaluate the effects on other target pathogens and quality features, overall data indicate that cold atmospheric plasma can be a promising technology towards safer and more environmentally sustainable food decontamination procedures.

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Plasma activated water as alternative decontamination treatment for leafy vegetables and wash water.

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Decontamination of wash water and fresh produce is generally achieved through chemicals being sodium hypochlorite the most commonly used one. However, there are several concerns related to human health and environmental pollution which lead to search for alternative methods. The aim of this study was to test effectiveness of plasma-activated water (PAW) in decontaminating baby leaf vegetables and the wash waters. For the wash waters, challenge tests with 3 pathogens – *Salmonella* Enteritidis, *Listeria monocytgenes*, *Escherichia coli* – were carried out by considering 2 contamination levels, i.e. 2-3 and 6 Log CFU/ml. Fresh produce was washed with PAW in comparison to chlorinated water by addressing natural microbiota. Also a shelf-life study was performed to test the effects of the treatments on the surviving indigenous microbiota over refrigerated storage. Results on wash waters showed that the highest efficacy was achieved only when initial contamination values were at the lowest levels regardless the target pathogen. Data on the baby leaf lettuce indicated that mesophilic bacteria were significantly reduced after 2 min of washing with both PAW and chlorine. However, prolonging washing up to 20 minutes did not significantly ($P>0.05$) improve the effectiveness. No significant differences were found for the counts of *Enterobacteriaceae*, while psychrotrophic bacteria seem to be the most sensitive population to chlorinated water after a 2-min washing. After 12 days of storage at 4°C, the baby leaf lettuce washed with chlorine showed black areas and loss of tissue consistency, whereas the PAW-treated vegetables maintained the visible quality and appearance at acceptable levels. Overall, proper tuning of processing conditions is necessary for wash waters by considering their specific features and particularly the contamination level, while PAW appears to be a promising innovative technology for leafy vegetable decontamination.

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Bioaccessibility of phenolic compounds from a microencapsulated propolis extract by spray drying during in vitro gastrointestinal digestion.

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Propolis is a natural product that bees make from wax, salivary secretions and resinous material collected from the flowers and leaf buds of certain plants. Its composition consists mainly of resins and balms, wax, volatile oils, pollen, and organic and mineral substances. In addition, propolis contains a wide variety of bioactive compounds such as flavonoids, phenolic acids derivatives and other compounds as well as terpenes and terpenoids. These bioactive compounds present in propolis have several beneficial properties, such as anti-inflammatory, antioxidant and antifungal, among others. However, some of these compounds with bioactive properties are easily degraded. To prevent degradation, an encapsulation process can be used, this technique allows the cover and protection of bioactive compounds in a polymer matrix and the release these compounds into different parts of the gastrointestinal tract. In this context, the objective of this work was to evaluate the bioaccessibility of the phenolic profile of a propolis extract (PE) and different formulations of microencapsulated propolis extract by spray drying with inulin (PE-IN), inulin/sodium alginate (PE-IN /SA), inulin/pectin (PE-IN/P) and inulin/chitosan (PE-IN/CH) using the INFOGEST protocol and characterized by HPLC-ESI-QTOF-MS/MS.

Analysis of the phenolic profile in PE and microparticles yielded a total of 58 tentatively identified compounds, mainly phenolic acid derivatives and flavonoids. Digestion of the free extract resulted in the degradation of most compounds, which was minimized in all encapsulated formulations. These findings support the protection of microencapsulation with these polymers and their potential use as a powerful strategy to increase the bioaccessibility of propolis bioactive compounds, improving its health effects. Therefore, all the microparticles developed could be promising strategies to improve the stability of this bioactive extract in gastrointestinal conditions, enhancing its beneficial effect.

Impact of fermentation by lactic acid bacteria on the content of polar compounds with antioxidant and antidiabetic activity in guava leaves extracts

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Psidium guajava L. leaves contains valuable compounds that have reported antioxidant and antidiabetic properties. They could be used in several industries such as food or pharmaceutical. The aim of this research was studied the changes experimented in phenolic composition, antioxidant, and antidiabetic activities of the extract from guava leaves after lactic acid fermentation. *P. acidilactici* CECT 5765T, *P. pentosaceus* CECT 923, *La. plantarum* CECT 748T, *Le. brevis* CECT 5354, *Leu. mesenteroides* CECT 219T and *La. plantarum* CECT 9567-C4 fermentation got significant increment in phenolic compounds in comparison with unfermented guava leaves samples. The improvement could be related to insoluble phenolics extraction by certain segregated enzymes like β-glucosidase or decomposition of high molecular weight

compounds into compounds with reduced molecular weight. The major increment in phenolic acids was observed with *La. plantarum* CECT 748T and *La. plantarum* CECT 9567-C4 fermentation whereas other fermentations enhanced the amounts of flavanones. Fermented samples with *P. acidilactici* CECT 5765T experimented the major increase being about 68% in polar compounds, almost 20% in alpha-amylase inhibition and nearly 18% in antioxidant activity. Antioxidant activity and alpha-amylase inhibition had a positive statistical ($p < 0.05$) Pearson correlation with polar compounds.

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Non-targeted authentication of monofloral honey by dielectric barrier discharge ionization high resolution mass spectrometry (DBDI-HRMS)

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Monofloral honey (MH) is a high quality honey which has a distinctive flavor due to its being generated predominantly from the nectar of a single plant species and it has beneficial effects for health. Unfortunately, the authenticity of the botanical origin, stated on the label of honey jar, a long-standing concern. The reference methods for the verification of the botanical origin of the honey are the melissopalynological analysis. The technique is laborious, expensive and needs well-trained operators. Therefore, to protect the consumers and beekeepers from the risks associated the deliberate/intentional frauds performed to obtain financial gain it becomes essential to set up reliable, cost-effective methods that authenticate the MH. The goal of this study is the development and validation of a dielectric barrier discharge ionization high resolution mass spectrometry (DBDI-HRMS) method for the authentication of the botanical origin of MH based on its volatile profile. A total of 112 certified samples from 7 different botanical origins (acacia, citrus, chestnut, sunflower, dandelion, linden and rhododendron) by three different operators. The first batch of samples included 64 samples and the second independent batch was comprised of 48 samples. After warming up at 40°c for 20 min in a thermostat, the honey samples were subsequently analyzed by DBDI-HRMS using a Sicrit® source (Plasmion gmbh, Germany) coupled to an Exactive Orbitrap mass spectrometer (Thermo fisher scientific) by opening the lid of the jar in front of the source for 6 seconds. The repeatability of the omics profiles is an open question. Therefore, we assessed the repeatability of the two spectral repetitions of each sample by cosine similarity and demonstrated the good repeatability of the analysis. The spectral data were normalized and three different classifiers were built up. The random forest classifier achieved the best performances with an accuracy of 100% and 82% in cross-validation and validation with independent batch and inexperienced operator, respectively. Sensitivity, specificity, area under the curve and Kappa statistics were calculated. The overfitting of the model was excluded by running permutations tests. This study shows encouraging results that could open a new avenue for the rapid and accurate authentication of MH.

Applied genomics in dairy science: from animal breeding to dairy product authentication.

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Genomics has changed dairy science introducing new technologies and new approaches in different steps from the value chain, that goes from the breeding and selection of the cattle to the authentication of dairy products. We have applied high-throughput genotyping approaches and whole genome sequencing to characterise the genetic variability of cattle of different breeds and obtain information on genomic regions containing major genes affecting milk quality and production traits, including casein genes. The obtained genomic information has been used to verify or reconstruct the pedigree of some cattle populations and then to redesign optimum contribution selection programs in local breeds. For two cattle breeds (Reggiana and Modenese), we obtained genomic information from almost all animals registered to their herd books. Signature of selection have been also identified in several cattle breeds by combining runs of homozygosity, F statistics and other population genetic approaches. These applications have made it possible to manage the cattle populations and re-define breeding and conservation programs of cattle genetic resources. Then authentication approaches based on DNA analyses have been set up to recover the breed of origin of dairy products, including Parmigiano-Reggiano cheese. Here, targeted PCR- based assays and untargeted next generation sequencing approaches have been set up for monitoring frauds derived from the use of milk of undeclared breeds or species.

Fruit juice beverages enriched with an innovative ingredient from clementine by-product.

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Nowadays, the organic waste and by-products generated during food processing represents a great challenge for institutional authorities and researchers both from an economic and environmental point of view. On the other hand, they are rich in substances with high added value. From a circular economy perspective, maximising the use of food resources also through the valorisation and reuse of by-products can reduce problems in waste management and environmental pollution. The aim of this study was to develop a beverage with added an innovative ingredient derived from clementine juice processing by-product. The formulation was based on a blend of ACE juices which was enriched with the innovative ingredient. In particular, the ingredient was produced through a biotechnological process based on a non-thermal pre-treatment - Pulsed Electric Field (PEF) – and microbial fermentation of the clementine by-product added with water by a selected strain of Saccharomyces cerevisiae. Then, different formulations of the ACE drink, based on orange, carrot and lemon juices, were prepared by supplementing the fermented clementine by-product at different contents. Overall, 9 beverages were prepared and characterised for β-carotene, vitamin C, total phenolic content (TPC) and volatile compounds (GC/MS-SPME analysis) in comparison to the control one (not supplemented with the innovative ingredient). Overall, the beverages presented improved functional properties compared to the control drink being the TPC and β-carotene contents strongly increased in the relation to the amount of innovative ingredient added. Based on the aroma profile, the ACE drink enriched with the fermented citrus by-product showed also higher contents of terpene compounds, such as β-carene, terpineol, cymene. In conclusion the use of by-products for food purposes appears to be a promising strategy for their valorisation and cost reduction for their management as wastes.

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