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4th International Conference on

FOOD BIOACTIVES & HEALTH

18.–21. 9. 2023

PRAGUE CONGRESS CENTRE
CZECH REPUBLIC

ABSTRACT BOOK

4TH FBHC
PRAGUE 2023
CZECH REPUBLIC

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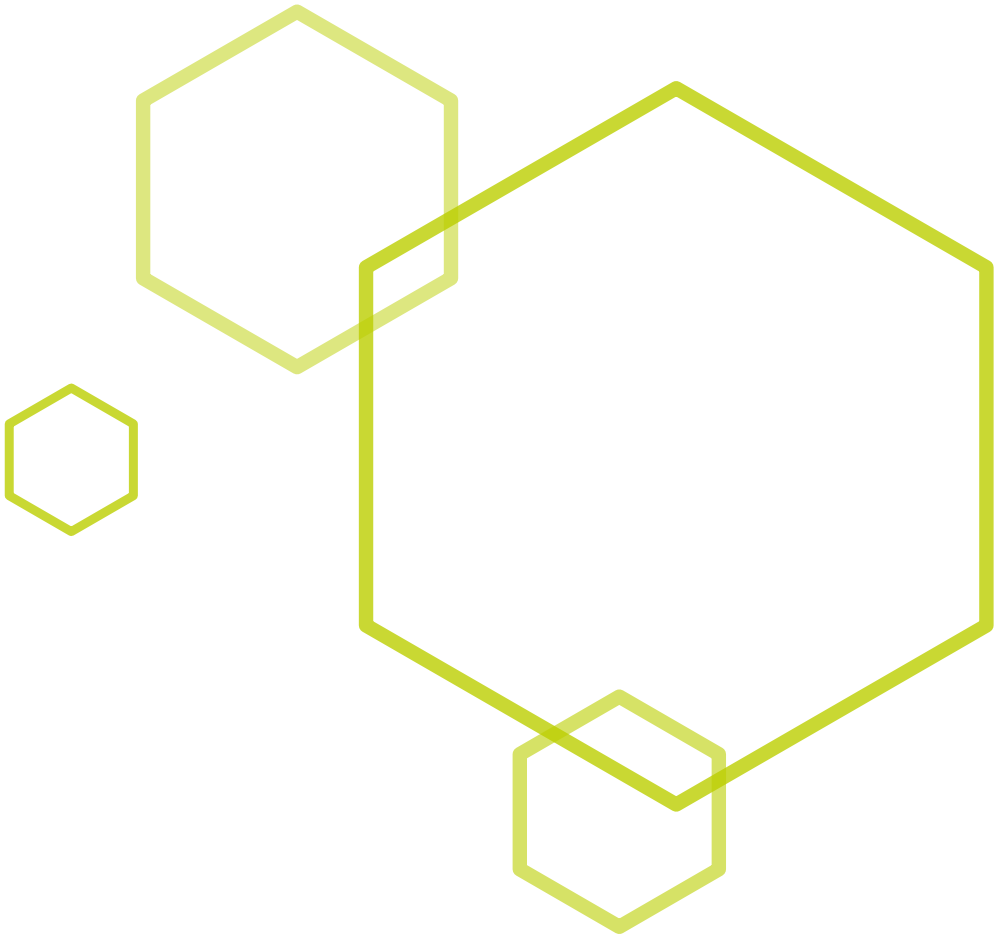


Monday, 18 September 2023

Welcome/Opening Ceremony

15:00–15:30 **Forum Hall**

Kateřina Valentová (Czech Republic), Jiří Hašek (Czech Republic)

Plenary Session I.

15:30–17:00 **Forum Hall**

Chairs: Kateřina Valentová (Czech Republic), Paul Kroon (United Kingdom)

15:30 **EXTRA! EXTRA! (POLY)PHENOLS FOR PERSONALISED AND PRECISION NUTRITION!!** O 001
Pedro Mena (Italy)

16:00 **OLD AND NEW POSTPRANDIAL BIOMARKERS TO MEASURE THE EFFECTS OF PHYTOCHEMICALS** O 002
Gary Williamson (Australia)

16:30 **DIETARY FACTORS INFLUENCING CAROTENOID BIOAVAILABILITY – FOCUS ON INTERACTIONS WITH DIETARY PROTEINS** O 003
Torsten Bohn (Luxembourg)

Welcome Drink

17:00–18:00

Tuesday, 19 September 2023

Plenary Session II.

08:30–09:45 **Forum Hall**

Chairs: Jaroslav Havlík (Czech Republic), Daniele Del Rio (Italy)

08:30 **A PLATFORM FOR SCREENING OF BACTERIAL ANTIBIOTIC RESISTANCE MODULATORS** O 004
Jitka Viktorová (Czech Republic)

08:55 **METABOLISM OF FOOD BIOACTIVES BY GUT MICROBIOTA MODULATES THEIR HEALTH EFFECTS** O 005
Francisco A. Tomás-Barberán (Spain)

09:20 **DISTINCT BIOLOGICAL ACTIVITIES AND HEALTH IMPACTS OF CITRUS LIMONOIDS WITH DIFFERING STRUCTURES**
Bhimu Patil (USA)

O 006

Industry Symposium: Sponsor's slot – Fluid Air

09:45–10:00 **Forum Hall**

STABILITY OF FOOD ACTIVE INGREDIENTS USING ELECTROSTATIC SPRAY DRYING TECHNOLOGY
Elodie Beaupeux (France)

Coffee Break/Poster Viewing

10:00–10:30

Plenary Session III.

10:30–12:00 **Forum Hall**

Chairs: Pedro Mena (Italy), Chris Gill (United Kingdom)

10:30	WHAT EVIDENCE FOR THE CONTRIBUTION OF FLAVANONES IN THE HEALTH EFFECTS OF CITRUS FOODS? Christine Morand (France)	O 007
11:00	ASSESSMENT OF DIETARY (POLY)PHENOL INTAKE: WHAT ARE WE MEASURING? Ana Rodriguez-Mateos (Spain)	O 008
11:30	MODULATION OF THE GUT MICROBIOTA AND IMMUNE SYSTEM BY DIET Miloslav Kverka (Czech Republic)	O 009

Lunch Break / Poster Viewing

12:00–13:30

Parallel Session: (Poly)Phenols I.

13:30–15:00 **Forum Hall**

Chairs: Christine Morand (France), Ana Rodriguez-Mateos (Spain)

13:30	NEW INSIGHTS INTO THE MICROBIOME-COCOA FLAVONOIDS INTERACTION IN AN *EX VIVO* COLONIC MODEL Sara Dobani (Italy)	O 010
13:45	NATURAL PRODUCT PHYTOCHEMICAL ANALYSIS USING A COMBINED FINGERPRINTING AND CHEMOMETRIC APPROACH Ance Bārzdiņa (Latvia)	O 011
14:00	BLOOD-BRAIN BARRIER TRANSPORT OF BIOACTIVES FROM MEDITERRANEAN FOODS María Carmen García-Parrilla (Spain)	O 012
14:15	MODULATORY EFFECT OF EXTRA VIRGIN OLIVE OIL POLYPHENOLS AND THEIR METABOLITES ON INFLAMMATORY RESPONSE IN INTESTINAL CACO-2 AND ENDOTHELIAL HUVEC CELLS Gabriele Serreli (Italy)	O 013
14:30	CHRONIC AND POSTPRANDIAL EFFECT OF BLUEBERRIES ON COGNITIVE FUNCTION, ALERTNESS AND MOOD IN PARTICIPANTS WITH METABOLIC SYNDROME – RESULTS FROM A 6-MONTH, DOUBLE BLIND, RANDOMIZED CONTROLLED TRIAL Peter Curtis (United Kingdom)	O 014
14:40	PROANTHOCYANIDINS MODULATE CENTRAL CLOCK, RESTORE DIURNAL RHYTHMICITY, AND IMPROVE CARDIOMETABOLIC RISK FACTORS IN CAF DIET-INDUCED OBESE RATS IN A TIME-OF-DAY-DEPENDENT MANNER Jorge Ricardo Soliz Rueda (Spain)	O 015
14:50	FROM DIET TO NEURONAL CELLS: INVESTIGATING THE EFFECTS OF LOW-MOLECULAR WEIGHT (POLY) PHENOL METABOLITES ON PARKINSON'S DISEASE Rafael Carecho (Portugal)	O 016

Parallel Session: Bioactive Peptides & Proteins

13:30–15:00 **South Hall 2B**

Chairs: Ryszard Ostaszewski (Poland), Bhimu Patil (USA)

13:30	A MILK PROTEIN HYDROLYSATE DECREASES INFLAMMATION ON A MODEL OF mBSA-INDUCED ARTHRITIS MICE Joanna Moro (France)	O 017
13:45	MEMBRANE FILTRATION PROCESSING OF INFANT MILK FORMULA PROMOTES GUT MATURITY *IN VITRO* AND *IN VIVO* Cathal Dold (Ireland)	O 018
14:00	OAT PROTEIN MODULATES CHOLESTEROL LEVELS AND IMPROVES CARDIAC SYSTOLIC FUNCTION IN HIGH-FAT, HIGH-SUCROSE FED RATS Sijo Joseph Thandapilly (Canada)	O 019
14:15	BIOACPEPFINDER: NOVEL BIOINFORMATICS TOOL FOR THE DISCOVERY OF BIOACTIVE PEPTIDES FROM PROTEIN DIGESTION Carlos Bathich (France)	O 020
14:30	EFFECTS OF INDIVIDUAL AMINO ACIDS ON APOA-I MRNA PRODUCTION AND PRO-APOA-I SECRETION Willem Zwaan (Netherlands)	O 021
14:45	BIOACTIVE PEPTIDES FROM MILK CONSUMPTION: POSTPRANDIAL CIRCULATING PROFILES AND IMPACT ON GASTROINTESTINAL DISCOMFORTS IN HEALTHY SUBJECTS Paola Vitaglione (Italy)	O 022

Coffee Break / Poster Viewing

15:00–15:30

Parallel Session: (Poly)Phenols II.

15:30–17:00 **Forum Hall**

Chairs: Gary Williamson (Australia), Vladimír Křen (Czech Republic)

15:30	APOE GENOTYPE, THE GUT MICROBIOTA AND POLYPHENOL METABOLISM INTERACTIONS Thomas Hunt (United Kingdom)	O 023
15:45	CAN PHASE II METABOLITES OF HESPERIDIN EXPLAIN THE BENEFICIAL EFFECTS OF ORANGE FLAVANONE ON VASCULAR ENDOTHELIAL FUNCTION? Laurent-Emmanuel Monfoulet (France)	O 024
16:00	SPIRULINA PROTEIN-POLYPHENOL PARTICLES ATTENUATE POLLUTION-INDUCED SKIN DAMAGE: A NOVEL SUSTAINABLE APPROACH Roberta Hoskin (USA)	O 025
16:15	THE EFFECT OF ANTHOCYANIN RICH PLUM ON LEARNING AND INFLAMMATION: ROLE OF IL-6 Naomi May (Australia)	O 026
16:30	MENTAL HEALTH IN NEW PARENTS: A RANDOMISED CONTROL TRIAL INVESTIGATING DIETARY FLAVONOID INTAKE AND MENTAL HEALTH IN THE POSTPARTUM PERIOD Rebecca Colombage (United Kingdom)	O 027
16:40	THE BATTLE OF SYNBIOTIC TREATMENTS AGAINST CELIAC DISEASE. A ROAD MAP VIEW IN DIETARY COMPOUNDS-PROBIOTIC INTERACTIONS Rosa Pérez-Gregorio (Spain)	O 028
16:50	UNRAVELLING THE ANTI-ANGIOGENIC EFFECT OF DIETARY ISOFLAVONES AND THEIR CIRCULATING METABOLITES IN HUMAN AORTIC ENDOTHELIAL CELLS Juan Antonio Gimenez Bastida (Spain)	O 029

Parallel Session: Gut Microbiota

15:30–17:00 **South Hall 2B**

Chairs: Yves Desjardins (Canada), Antonio González-Sarrías (Spain)

15:30	CHARACTERIZATION OF THE INTER-INDIVIDUAL VARIABILITY ASSOCIATED WITH THE MICROBIAL METABOLISM OF (−)-EPICATECHIN Jacob Lessard-Lord (Canada)	O 030
15:40	CITRUS EXTRACT HIGH IN FLAVONOIDS BENEFICIALLY ALTERS GUT MICROBIOTA METABOLIC RESPONSES IN HEALTHY SUBJECTS WITH FEATURES OF METABOLIC SYNDROME Yala Stevens (Netherlands)	O 031
15:50	PREVIOUS VAGINAL DELIVERY OR BREASTFEEDING AND DIETARY STARCH AND AMINO ACIDS HAVE THE STRONGEST ENVIRONMENTAL ASSOCIATIONS WITH THE VAGINAL MICROBIOME IN PREGNANCY; SECONDARY ANALYSIS OF THE MICROBEMOM RANDOMISED CONTROLLED TRIAL Gillian Corbett (Ireland)	O 032
16:00	REFINED DIETS ALTER BILE ACID PROFILES IN THE GUT AND THE BRAIN AND ARE CONCURRENT WITH INCREASES IN NEUROINFLAMMATORY SIGNALLING Emily Connell (United Kingdom)	O 033
16:10	ANTI-INFLAMMATORY POTENTIAL OF A SUPERCRITICAL FLUID EXTRACT FROM CHICORY ON THE INTESTINAL MUCOSA: IMPLICATIONS OF GUT MICROBIOTA METABOLIZATION Melanie Matos (Portugal)	O 034
16:20	IDENTIFYING NOVEL BIOLOGICAL ACTIONS OF THE MANGOSTEEN (*GARCINIA MANGOSTANA*) IN PROSTATE AND COLON CANCER Jeremy Johnson (USA)	O 035
16:30	DEVELOPMENT OF A NOVEL (POLY)PHENOL-RICH DIET SCORE AND ITS ASSOCIATION WITH URINARY (POLY)PHENOL METABOLITES Yong Li (United Kingdom)	F 001
16:33	4-METHYLCATECHOL IS AN ACTIVE ANTIPLATELET DRUG IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS Lukáš Konečný (Czech Republic)	F 002
16:36	UPSCALING APPLEWOOD EXTRACT PRODUCTION: ULTRASOUND ASSISTED EXTRACTION FROM LAB TO SEMI-INDUSTRIAL SCALE Hannes Withouck (Belgium)	F 003
16:39	GRAPE SEED PROANTHOCYANIDIN EXTRACT (GSPE) COULD IMPROVE METABOLIC SYNDROME SYMPTOMS IN LIVER BY MODULATING CIRCADIAN RHYTHMS OF ANTIOXIDANT-RELATED PARAMETERS IN A TIME-OF-DAY DEPENDENT MANNER Antonio Jesús Cortés Espinar (Spain)	F 004
16:42	INVESTIGATION OF ENDOGENOUS AND/OR EXOGENOUS PHENOLIC METABOLITES IN HUMANS USING (UN)TARGETED METABOLOMICS (ENDOPHENOL) Laila Guimarães Zeraik Cardoso (Italy)	F 005
16:45	EXPLORING THE POTENTIAL OF HYDROXYTYROSOL AS AN ADJUVANT AGENT IN COLORECTAL CANCER TREATMENT: EFFECTS OF ITS COMBINATION WITH CHEMOTHERAPY DRUGS ON LOVO SPHEROIDS Ana Catarina Macedo (Portugal)	F 006
16:48	LIGNAN-DERIVED METABOTYPES AND THEIR ASSOCIATION WITH THE CARDIOMETABOLIC HEALTH STATUS Maria Sole Morandini (Italy)	F 007
16:51	BARLEY-BASED DIETS RICH IN BIOACTIVE COMPOUNDS ENHANCE GUT HEALTH THROUGH MODULATION OF MICROBIOTA AND INFLAMMATION María Engracia Cortijo Alfonso (Spain)	F 008

Poster Session

17:00–19:00 **Forum Hall**

Wednesday, 20 September 2023

Plenary Session IV.

08:30–09:45 **Forum Hall**

Chairs: Aedin Cassidy (United Kingdom), Jitka Ulrichová (Czech Republic)

08:30 BLACK SOLDIER FLY LARVAE AS A NEW SUSTAINABLE SOURCE OF DIETARY BIOACTIVES O 036
Patrick Borel (France)

09:00 MYCOTOXINS IN OUR DIET: DEEPENING THE KNOWLEDGE MINIMIZES THE HEALTH RISK (?) O 037
Milena Stránská (Czech Republic)

09:30 PYRROLIZIDINE ALKALOIDS IN *BORAGO OFFICINALIS*: PROFILING AND QUANTIFICATION O 038
Melinda Sattler (Germany)

Industry Symposium: Sponsor's slot – Bioactor

09:45–10:00 **Forum Hall**

SHORT-TERM *ARONIA MELANOCARPA* EXTRACT SUPPLEMENTATION IMPROVES COGNITIVE PERFORMANCE: A RANDOMIZED CONTROLLED TRIAL IN HEALTHY YOUNG ADULTS O 039
Sanne Ahles (Netherlands)

Coffee Break / Poster Viewing

10:00–10:30

Plenary Session V.

10:30–11:45 **Forum Hall**

Chairs: Anika Wagner (Germany), Pavla Bojarová (Czech Republic)

10:30 PRODUCTION AND EVALUATION OF RARE SUGARS AND GLYCOSIDES AS NEW FOOD INGREDIENTS O 040
Tom Desmet (Belgium)

10:55 PROTOCOLS FOR INVESTIGATING OF THE PRODUCTION OF COLONIC CATABOLITES OF DIETARY (POLY) PHENOLS O 041
Alan Crozier (Saudi Arabia)

11:20 MOLECULAR EFFECTS OF SILYMARIN FLAVONOLIGNANS: CHIRALITY IS PIVOTAL IN BIOLOGICAL ACTIVITY O 042
Vladimír Křen (Czech Republic)

Industry Symposium: Sponsor's slot – SilvaTeam

11:45–12:00 **Forum Hall**

INNOVATIVE APPLICATIONS OF NATURAL TANNIN EXTRACTS IN THE FOOD INDUSTRY
Giulia Potenziani (Italy)

Lunch Break / Poster Viewing

12:00–13:30

Parallel Session: (Poly)Phenols III., Bioavailability, Absorption, Distribution, Metabolism & Excretion

13:30–15:00 **Forum Hall**

Chairs: Přemysl Mladěnka (Czech Republic), Letizia Bresciani (Italy)

13:30	USE OF *CALLISTEMON CITRINUS* EXTRACTS FOR THE PRODUCTION OF BIOACTIVE PACKAGING Marika Avitabile (Italy)	O 043
13:45	IMPACT OF FOOD STRUCTURE ON THE BIOACCESSIBILITY OF BERRY (POLY)PHENOLS Zicheng Zhang (United Kingdom)	O 044
14:00	ASSESSING THE IMPACT OF A (POLY)PHENOL-RICH DIET ON BIOMARKERS OF INTESTINAL PERMEABILITY AND INFLAMMATION IN OLDER ADULTS: THE MaPLE RANDOMIZED CONTROLLED TRIAL Mirko Marino (Italy)	O 045
14:15	CHARACTERIZATION OF THE URINARY PROFILE OF PHENOLIC METABOLITES OF POSTMENOPAUSAL WOMEN AFTER DIET SUPPLEMENTATION WITH CHOCOLATE, GREEN TEA AND FRUIT JUICE Lorena Sánchez Martínez (Italy)	O 046
14:30	EVALUATION OF URINARY PHENYL-VALEROLACTONES AS BIOMARKERS OF DIETARY FLAVAN-3-OL EXPOSURE Benjamin Parmenter (Australia)	O 047
14:45	POLYPHENOL OXIDASE AND BIOAVAILABILITY OF FLAVAN-3-OLS FROM FRUIT SMOOTHIES: IMPORTANCE FOR FOOD PREPARATION AND DIETARY ADVICE Javier Ottaviani (USA)	O 048

Parallel Session: Carotenoids, Glucosinolates, Fatty Acids & Sterols

13:30–15:00 **South Hall 2B**

Chairs: Torsten Bohn (Luxembourg), Patrick Borel (France)

13:30	EXAMINING WAYS TO IMPROVE SLEEP QUALITY AND SUPPORT HEALTHY AGEING IN OLDER ADULTS WITH SLEEP DISTURBANCES THROUGH TARGETING THE GUT MICROBIOME WITH SAFFRON SUPPLEMENTATION Leonie Lang (United Kingdom)	O 049
13:45	RESEARCH ON CAROTENOIDS IN THE HEALTH-PROMOTING AND SUSTAINABLE FOODS ERA Antonio J. Meléndez-Martínez (Spain)	O 050
14:00	LONGITUDINAL STUDY OF THE ASSOCIATION BETWEEN TOMATO CONSUMPTION AND BLOOD PRESSURE IN AN OLDER POPULATION AT HIGH CARDIOVASCULAR RISK David Murcia Lesmes (Spain)	O 051
14:10	HUMAN INTERVENTION STUDY TO ASSESS THE URINARY EXCRETION OF ORGANOSULFUR COMPOUNDS AFTER ACUTE INTAKE OF BLACK GARLIC Alicia Moreno-Ortega (Spain)	O 052
14:20	DETERMINATION OF BROCCOLI (*BRASSICA OLERACEA* VAR. *ITALICA*) GLUCOSINOLATE CONTENT USING MID-INFRARED (MIR) SPECTROSCOPY COUPLED WITH CHEMOMETRICS Faye Langston (United Kingdom)	O 053
14:30	ALLYL-ISOTHIOCYANATE AFFECTS THE ENERGY METABOLISM OF *DROSOPHILA MELANOGASTER* FED A HIGH SUGAR DIET Anika Wagner (Germany)	O 054
14:40	ASSOCIATIONS BETWEEN NON-CHOLESTEROL STEROLS, ASTHMA SEVERITY, AND AIRWAY INFLAMMATION IN PEDIATRIC POPULATIONS: THE ADEM1 AND MIKADO STUDIES Lieve Van Brakel (Netherlands)	O 055

14:50 POSTPRANDIAL HYPERLIPIDEMIA MODULATES HIGH DENSITY LIPOPROTEIN CHOLESTEROL EFFLUX CAPACITY IN A FATTY ACID-DEPENDENT MANNER O 056
Elena Grao-Cruces (Spain)

Coffee Break / Poster Viewing

15:00–15:30

Parallel Session: Functional Foods & Food Supplements

15:30–17:00 **Forum Hall**

Chairs: Jitka Viktorová (Czech Republic), Paola Vitaglione (Italy)

15:30 A PROBIOTIC FUNCTIONAL FOOD ENRICHED IN PHYTOSTEROLS AND CAROTENOIDS TO TARGET HYPERCHOLESTEROLEMIA, INSULIN RESISTANCE, VITAMIN A STATUS AND GUT MICROBIOTA IN HIGH FAT DIET-INDUCED METABOLIC SYNDROME RATS O 057
Claudie Dhuique-Mayer (France)

15:45 MASTIHA OIL EXHIBITS FAVORABLE EFFECTS IN METABOLICALLY UNHEALTHY ADULTS – A RANDOMIZED CONTROLLED TRIAL O 058
Evdokia Valsamidou (Greece)

16:00 SOURCE-SPECIFIC NITRATE INTAKE AND INCIDENT DEMENTIA IN THE DANISH DIET CANCER AND HEALTH STUDY COHORT O 059
Catherine Bondonno (Australia)

16:15 POTENTIAL OF OAT-DERIVED COMPONENTS IN THE PREVENTION OF HYPERTENSION AND ITS CARDIOVASCULAR COMPLICATIONS O 060
Thomas Netticadan (Canada)

16:30 VALORIZATION OF ENDIVE CO-PRODUCTS FOR FUNCTIONAL FOOD APPLICATIONS O 061
Pauline Bruniaux (France)

16:40 FIBRE AND PREBIOTIC SUBSTANCES FROM STARCH IN HUMAN NUTRITION O 062
Janusz Kapusniak (Poland)

16:50 EXPLORING THE EFFECT OF A MULTI-SPECIES PROBIOTIC ON COGNITIVE FUNCTION AND MOOD IN HEALTHY OLDER ADULTS, AND AN EXPLORATION INTO MICROBIALLY-DERIVED METABOLITES AS A MECHANISM OF ACTION O 063
Jessica Eastwood (United Kingdom)

Parallel Session: Personalized Nutrition

15:30–17:00 **South Hall 2B**

Chairs: Alan Crozier (Saudi Arabia), Francisco A. Tomás-Barberán (Spain)

15:30 DEVELOPMENT OF A PLATFORM FOR THE ANALYSIS OF BIOMARKERS OF FOOD INTAKE WITHIN THE EU H2020 PREVENTOMICS PROJECT O 064
Claudia Favari (Italy)

15:40 SYSTEMATIC LITERATURE-BASED VALIDATION OF BIOMARKERS OF FOOD INTAKE FOR MULTIPLE PLANT FOODS O 065
Christoph Hassenberg (Germany)

15:50 THE IMPACT OF HIGH-FAT KETOGENIC DIET AND LOW-FAT DIET IN THE BODY WEIGHT AND CARDIOVASCULAR RISK FACTORS ON OVERWEIGHT AND OBESE SAUDI WOMEN: A RANDOMIZED CONTROLLED TRIAL O 066
Randah Alqurashi (Saudi Arabia)

16:00 MULTIMETHOD APPROACH TO DETERMINE DIETARY BIOMARKERS IN HUMAN URINE USING LC-MS/MS ANALYSIS SIMULATING CONDITIONS CLOSE TO EVERYDAY LIFE O 067
Amelie Frank (Germany)

16:10 A SYSTEMATIC REVIEW OF FACTORS AFFECTING THE INTER-INDIVIDUAL VARIABILITY IN THE PRODUCTION AND BIOAVAILABILITY OF (POLY)PHENOLIC METABOLITES O 068
José Fernando Rinaldi De Alvarenga (Italy)

16:20 SPECIFIER PROTEIN AND MYROSINASE ACTIVITY AND GLUCOSINOLATE PROFILE DETERMINE THE OUTCOME OF GLUCOSINOLATE HYDROLYSIS PRODUCT FORMATION AND PROFILE IN KOHLRABI TISSUES F 009
Kudzai Gracious Mbudu (Germany)

16:23 PHYTOCHEMICAL CHARACTERIZATION AND VOLATILE COMPOSITION OF 38 SAFFRON (*CROCUS SATIVUS* L.) STIGMAS PRODUCED IN ALGERIA: IDENTIFICATION OF COMPOUNDS WITH ANTIOXIDANT EFFECT F 010
Leonor Teixeira Da Costa (Portugal)

16:26 PROTEIN-PHENOL INTERACTION AS A STRATEGY TO ELIMINATE THE IMMUNOGENIC GLUTEN PEPTIDES F 011
Merve Aksoy (Turkey)

16:29 ROLE OF GUT MICROBIOTA IN THE ANTIHYPERTENSIVE EFFECT OF PROTEIN HYDROLYSATES IN SHR F 012
Rafael Ángel López-Villalba (Spain)

16:32 A COMPARATIVE STUDY OF EFFECTS OF INULIN AND CHICORY ON GUT HEALTH IN PIGLETS DURING THE WEANING PERIOD F 013
Tushar Kulkarni (France)

16:38 IMPACT OF REDUCED-PHOSPHATE-AVAILABILITY ON ESSENTIAL MICRONUTRIENTS IN MAIZE FOR HUMAN CONSUMPTION F 015
Esteban Gutierrez La Torre (Germany)

16:41 EFFECT OF PROCESSING METHOD ON HEAVY METAL CONTENT IN SPECIALTY COFFEE F 016
Matus Varady (Slovakia)

16:44 NUTRITIONAL, FUNCTIONAL AND SAFETY COMPARISON OF AN AUSTRALIAN NATIVE GRAIN WITH WHOLE WHEAT F 017
Luke Williams (Australia)

16:47 ANTI-INFLAMMATORY EFFECT OF POLAR AND NON-POLAR EXTRACTS OF AFRICAN GREEN LEAFY VEGETABLES ON LPS-STIMULATED THP-1 MACROPHAGES F 018
Nelly Fioroni (France)

16:50 INVESTIGATING THE BIOACTIVE PROPERTIES OF APPLE POMACE: UNLOCKING ITS POTENTIAL F 019
Liege Aguiar Pascoalino (Portugal)

16:53 PRECISION NUTRITION TO IMPROVE CARDIOMETABOLIC HEALTH WITH DIETARY (POLY)PHENOLS (PRE-CARE-DIET): A RESEARCH PROTOCOL F 020
Cristiano Negro (Italy)

Conference Dinner

19:00–22:00

Thursday, 21 September 2023

Plenary Session VI.

09:00–10:00 **Forum Hall**

Chairs: Patrizia Riso (Italy), Mi Kyeong Lee (Republic of Korea)

09:00 FLAVONOIDS AND THEIR METABOLITES AS POTENTIAL SOURCES OF NOVEL ANTIPLATELET DRUGS O 069
Přemysl Mladěnka (Czech Republic)

09:30 NEW ANALYTICAL CHALLENGES TO EVALUATE (POLY)PHENOL METABOLISM AND BIOACTIVITY
Letizia Bresciani (Italy) O 070

Coffee Break / Poster Viewing

10:00–10:30

Plenary Session VII.

10:30–12:00 **Forum Hall**

Chairs: David Vauzour (United Kingdom), Sonia De Pascual-Teresa (Spain)

10:30 HEALTH BENEFITS OF DIETARY POLYPHENOLS THAT ARE DRIVEN BY THEIR INTERACTIONS THE GUT MICROBIOTA O 071
Paul Kroon (United Kingdom)

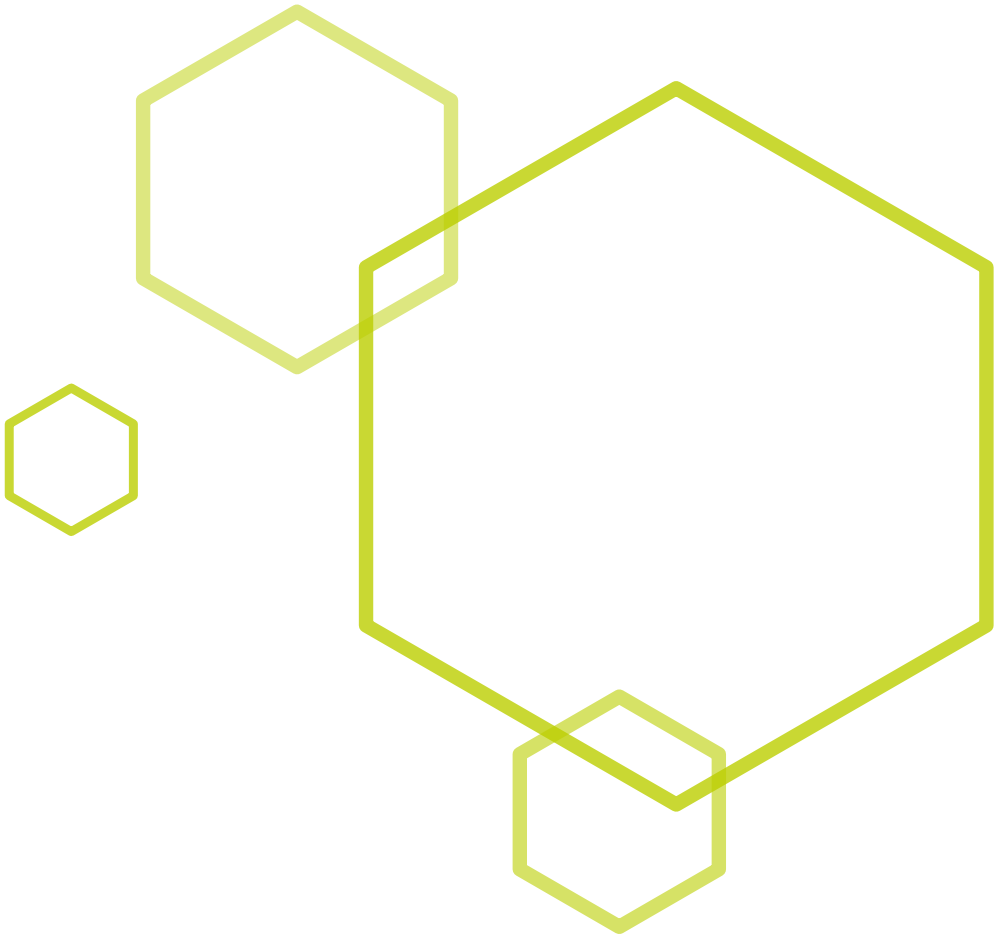
11:00 GUT IT ON: HOW MICROBIAL (POLY)PHENOLS METABOLITES KEEP YOUR BRAIN INFLAMMATION-FREE O 072
Claudia Nunes dos Santos (Portugal)

11:30 CRANBERRY PROCYANIDINS AID IN MAINTAINING A HEALTHY GUT BY PROMOTING A FAVORABLE GUT MUCOSAL ENVIRONMENT AND A UNIQUE INTERACTION BETWEEN THE MICROBIOTA AND THE HOST O 073
Yves Desjardins (Canada)

Closing Ceremony and Awards

12:00–13:00 **Forum Hall**

Oral Presentations



O 001 EXTRA! EXTRA! (POLY)PHENOLS FOR PERSONALISED AND PRECISION NUTRITION!!

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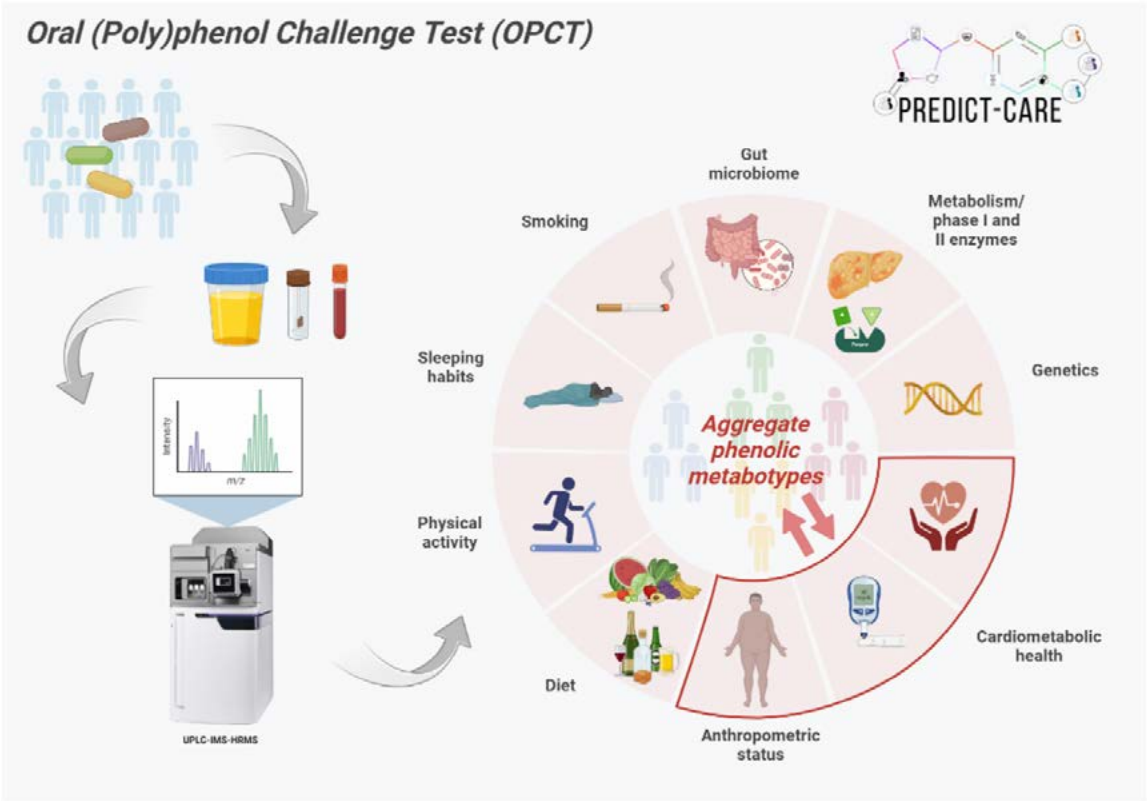
Hot news!

The scientific community is disentangling the factors conditioning the inter-individual variability in the metabolism and response to dietary (poly)phenols. But, are we ready to launch effective personalised dietary strategies? Let's discuss the topic!

Obesity, cardiometabolic diseases and neurological disorders represent serious healthcare issues at global level. Plant-based diets, rich in (poly)phenols and other bioactives, may be a solution, but their true efficacy may be compromised by the heterogeneity in phytochemical metabolism and the individual response to their consumption, as well as by the lack of studies relying on comprehensive experimental designs. (Poly)phenols are major plant bioactives metabolised into bioavailable molecules able to impact on different biological processes related to human health. However, the factors driving the inter-individual variability observed are somehow a major conundrum limiting the prospects of (poly)phenols for sound dietary strategies. But, times are changing: this individual heterogeneity, rather than being an obstacle, can pay the way for personalised approaches.

Thanks to some great studies, we are aware of the existence of metabotypes in the metabolism of some (poly)phenol classes. But we are lacking comprehensive information on the relationships among different types of (poly)phenols and different dietary patterns. Our group is trying to shed light on this. Among other initiatives, we have recently finished a 300-subject acute intervention with 15 different classes of dietary (poly)phenols, the Oral (Poly)phenol Challenge Test (OPCT), a study focused on the identification of aggregate phenolic metabotypes and the factors driving inter-person variability in the metabolism of (poly)phenols. Here we will be presenting the results of this study, estimating the contribution of the gut microbiota and genetic polymorphisms to (poly)phenol metabolism, and investigating the cardiometabolic health status of the individuals belonging to each metabotype. A complete individual profiling (Figure 1) serving as a starting point to develop tailored nutritional strategies with (poly)phenols.



Using the results of the previous study, our group is launching a precision nutrition study with (poly)phenols: PRE-CARE-DIET. This randomised, controlled trial will assess how a differential capacity to metabolise dietary (poly)phenols affects cardiometabolic health and obesity. A complete dietary assessment of food components, gut microbiota profiling, genotyping, and metabolic phenotyping will serve to understand the key determinants behind the heterogeneity in the individual's biological response to (poly)phenol consumption, adopting a comprehensive multi-omics approach and creating predictive models embracing the singularities of each individual. We hope this precision approach will serve to better understand the role of (poly)phenols and plant-based dietary patterns in improving cardio-metabolic health at individual level.

Fruitful discussion is expected considering the need to better understand the contribution of phytochemicals to the health effects of plant-based foods.

Funding sources:

The European Research Council (ERC) (PREDICT-CARE study, Grant Agreement N. 950050), the European Commission, NextGenerationEU (OBI-WAN-DIET, PNRR PE ON Foods, code PE00000003), and the Italian Ministry for Universities and Research (MUR) under the FARE programme (CARE-DIET, R20MPBW4FM).

O 002 OLD AND NEW POSTPRANDIAL BIOMARKERS TO MEASURE THE EFFECTS OF PHYTOCHEMICALS

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Consumption of meals high in fat and/or sugar result in an acute oxidative stress on the body. Regular and continued consumption of such meals leads to chronic inflammation and increased risk of metabolic diseases. Certain polyphenols and other phytochemicals, when consumed with food, can attenuate glucose spikes in the blood, but can also affect other biomarkers. We have developed experimental high fat/high sugar foods to generate post-prandial oxidative stress and assessed novel response biomarkers as targets of phytochemical action. The state of the art, current results and future research needs in this area will be presented.

O 003 DIETARY FACTORS INFLUENCING CAROTENOID BIOAVAILABILITY – FOCUS ON INTERACTIONS WITH DIETARY PROTEINS

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Background:

The intake of carotenoids from the diet as well as their circulating plasma concentrations have been inversely associated with the risk of several chronic diseases, including cardiovascular ones, diabetes, and some types of cancer. However, their absorption is usually low and quite variable, due to their lipophilicity. While it is known that a certain amount of fat is required to facilitate optimal bioaccessibility and absorption, other factors, such as competing lipophilic plant bioactives, dietary fiber, divalent minerals and also proteins have been studied to a far lesser extent.

Objectives:

This presentation strives to highlight some of these understudied aspects, with a focus on dietary proteins.

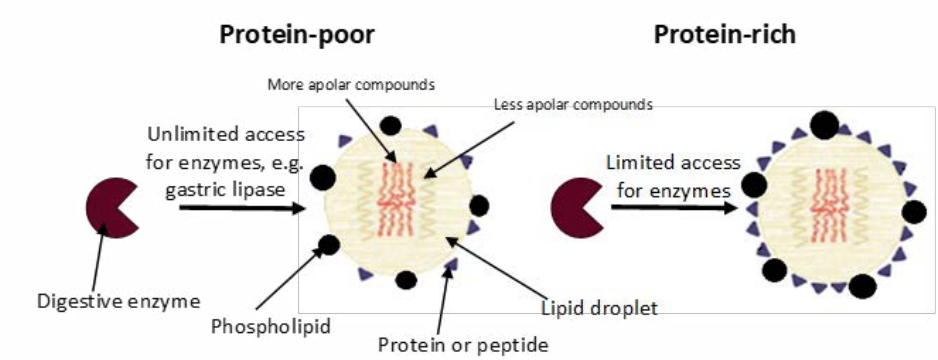
Methods:

Several studies were conducted to investigate the effects of proteins on relevant aspects of carotenoid bioavailability such as bioaccessibility, cellular uptake, and *in vivo* bioavailability in humans, and findings are discussed in the background of additional literature.

Results and discussion:

Whey protein isolate (WPI), gelatin, sodium caseinate and soy protein isolate (SPI) had received the main attention regarding their interactions with carotenoids, either in isolated form or studied within various food matrices. In a first *in vitro* study, it was shown that WPI modulated beta-carotene bioaccessibility depending on digestive conditions. While high simulated peristalsi and a reduced amount of co-digested lipids resulting in stronger positive effects on beta-carotene bioaccessibility (ca. 20%), high amounts of oil, lower bile salt concentration and lower amounts of pancreatin resulted in negative effects of WPI on beta-carotene bioaccessibility. In additional studies, co-digested proteins increased the bioaccessibility of individual carotenes, i.e. lycopene and beta-carotene, by almost 2-fold, while individual xanthophylls such as lutein were reduced in their bioaccessibility to about half. Similarly, for selected carotenoid-rich food matrices such as tomato juice, carrot juice and spinach, both bioaccessibility and cellular uptake were more enhanced for apolar carotenes vs. xanthophylls. More soluble and better digestible proteins also resulted in tendency in more positive effects on carotenoid bioaccessibility/cellular uptake, both studied in isolated form and from selected food matrices. Proteins from turkey and cod showed less strong effects, perhaps due to their presence in a more complex and stable food matrix. These findings were finally confirmed in a human postprandial randomized crossover trial (n=24 participants), in which a well soluble and digestible protein (WPI, ca. 30 g) vs. a less soluble and digestible one (SPI, ca. 30 g) improved by about 1/3 the absorption of individual and total carotenoids from the tomato/carrot juice as studied by the area under curve in the plasma-triacylglycerol rich fraction.

Figure 1: Action of proteins on enzymatic access on lipid droplets.



Conclusions:

In summary, while proteins could be a double-edged sword that could either increase or impede (Figure 1) several aspects related to carotenoid bioavailability, well digestible and soluble proteins have shown the potential to significantly increase the bioavailability of these health-associated pigments, likely via fostering lipid droplet processing into mixed micelles and fostering their stability.

O 004 A PLATFORM FOR SCREENING OF BACTERIAL ANTIBIOTIC RESISTANCE MODULATORS

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The development of new antibiotics does not keep up with the development of bacterial resistance. New antibiotics are developed only very slowly and are often only modifications of existing structures to which bacteria have already developed resistance. However, with the development of new molecular-biological methods, we are offered the possibility to more effectively and significantly faster identify the mechanisms that bacteria use to eliminate antibiotics.

The aim of this work is to prepare a platform for the search for inhibitors of antibiotic resistance. Clinical isolates of *Staphylococcus aureus* were first phenotypically and genotypically characterized from the point of view of sensitivity to antibiotics. Subsequently, the identified resistance determinants were introduced into genetically modified strains of *Escherichia coli* and *S. aureus* in an attempt to create a collection of strains where each strain would contain only one mechanism for eliminating the antibiotic. Using the collection created in this way, 300 compounds of different origins and structures were tested. The activity of compounds capable of synergistic action with the antibiotic was subsequently verified by determining the inhibition of enzyme or transport activity. The selected compounds were then tested on a collection of clinical isolates of different bacterial genera resistant to the given antibiotic to determine the selectivity of the given inhibitor.Preliminary results show that the platform created in this way can be used for high-throughput testing of potential adjuvant compounds for the treatment of drug-resistant infections.

The project was funded by the National Institute of Virology and Bacteriology (EXCELES Program, project LX22NPO5103) - Funded by the European Union - Next Generation EU and by the Czech Science Foundation (project No. 21-00551S).

O 005 METABOLISM OF FOOD BIOACTIVES BY GUT MICROBIOTA MODULATES THEIR HEALTH EFFECTS

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Most fruit and vegetable bioactives are phytochemicals, secondary metabolites for which the demonstration of their effects on human health has been complex in clinical trials. A large interindividual variability is observed that decreases the statistical significance of the results. The bioavailability of phytochemicals is often very low, and they reach the colon in significant concentrations, where they are metabolized by gut microbiota.

The metabolism of different plant-food bioactives from a variety of phytochemical groups (phenolics, terpenoids, nitrogen-containing, and sulphur-containing compounds) by human gut microbiota is reviewed, as well as their bioavailability and biological effects. The resident microbial metabolism produces many new metabolites in the gastrointestinal tract.

The metabolites produced are identified using metabolomic approaches, classical isolation, and structural determinations. The number of new metabolites identified has grown exponentially. Hydrolysis, oxidations, reductions (double bond hydrogenations), demethylations and methylations, ring cleavage, decarboxylations, and dehydroxylations are reactions produced by gut microbes on different food bioactives leading to small-size catabolic metabolites.

Identifying the gut bacteria responsible for food phytochemicals metabolism and the enzymes involved has also been a relevant scientific objective. Probiotics (Lactobacillaceae, Bifidobacteriaceae) and other bacteria belonging to the families Eggerthelaceae, Lachnospiraceae, and Bacteroidaceae have been demonstrated to be very active metabolizing food phytochemicals during colonic fermentation.

The produced metabolites have often shown better bioavailability than the parent compounds and significant health effects impacting gastrointestinal, muscular, cardiovascular, and neuronal functions. In some cases, there is robust *in vitro* evidence using the bioavailable metabolites and the concentrations found in the target tissues. Preclinical studies with different animal models and clinical trials considering the gut microbiota metabolites have also been introduced to demonstrate the health effects of the food-bioactive gut microbiota metabolites.

A large part of the interindividual variability in response to food bioactives can be attributed to differences in gut microbiota composition and metabolism. Different gut microbiota metabotypes have been described to explain the interindividual variability and the differences in the metabolites produced. The metabotypes need to be better defined, and accurate methods for their identification must be described and validated.

Stratification using metabotypes in clinical trials must be considered to correlate the response to the interventions with food bioactives with gut microbiota composition and the metabolites produced.

O 006 DISTINCT BIOLOGICAL ACTIVITIES AND HEALTH IMPACTS OF CITRUS LIMONOIDS WITH DIFFERING STRUCTURES

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[1] *Texas A&M University, Vegetable and Fruit Improvement Ctr, Department of Horticultural Sciences, College Station, USA*

Limonoids are a type of triterpenoid found in citrus fruits; these compounds have complex structures and are difficult to extract. The most commonly isolated limonoids from citrus fruits are limonin, nomilin, and obacunone, which exhibit anti-inflammatory, antioxidant, anti-cancer, and neuroprotective effects. Structural modifications to limonoids have resulted in novel compounds with enhanced biological activity. For example, modifying limonin to defuran limonin and limonin 7-methoxime enhances glutathione *S*-transferase (GST) activity and reduces inflammation when combined with curcumin treatment using cultured SW480 and 112CoN cells. The furan ring in limonoids plays an important role in their biological activity. Furan ring and C-7 substitutions in modified limonin inhibit cell-cell signaling and biofilm formation, while furan and an intact A-ring structure contribute to cancer prevention and anti-inflammatory responses.

To test the effect of limonoid structure on their function, we used parent limonoids and defuran derivatives by oxidative cleavage of furan ring. These alterations in functional groups of limonoids had different effects on p38 MAP kinase activity in human aortic smooth muscle cells which involves regulation of signal transduction pathway notably target for anti-inflammatory therapies. For the first time, we provided evidence that nomilin is a potent natural inhibitor of p38 MAP kinase activity in human aortic smooth muscle cells. These data also suggest that the seven-membered A ring with acetoxy group present in nomilin is essential for its inhibition of p38 MAP kinase.

Limonoids and their derivatives induced mitochondria-mediated intrinsic apoptosis in cultured SW480 cells and stimulated Phase II enzymes, indicating an anticarcinogenic effect, which requires further studies in related gut health models. Furthermore, the functional groups in limonoids influenced their interactions with gut microbiota and could potentially improve gut health or prevent illnesses. We will also discuss the potential of natural and modified limonoids as dietary supplements and therapeutic agents with a range of health benefits.

This research was funded by United States Department of Agriculture-NIFA-SCRI- 2017-51181-26834 through National Center of Excellence for Melon at the Vegetable and Fruit Improvement Center and Institute of Health through Agriculture of Texas A&M University.

O 007 WHAT SCIENCE-BASED EVIDENCES OF THE VASCULO-PROTECTIVE EFFECT OF DIETARY FLAVANONES?

Christine Morand[1]

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Well established evidence from meta-analysis of large prospective cohort studies indicate that an increased intake of total dietary flavonoids significantly reduced the incidence of cardiovascular diseases (CVD). Although fruits are recognized as major contributors to dietary flavonoid intake in humans, clinical studies focusing on the vascular health effects of the diversity of fruit flavonoids remain still limited compared to other food sources like cocoa, tea or soya. This is particularly true for the flavanone subclass, which is found specifically and abundantly in citrus-based foods, and for which however more and more results from preclinical studies are testifying to their vascular protective effects and suggesting associated mechanisms of action.

The present talk aims (i) to provide an overview of the recent state of the art related to the fate of dietary flavanones in human body and on the available knowledge on their physiological effects related to vascular protection, from data resulting of epidemiological, clinical and mechanistic research and (ii) to highlight the research questions that should be tackled more deeply to optimize the health benefit of flavanone consumption.

O 008 ASSESSMENT OF DIETARY (POLY)PHENOL INTAKE: WHAT ARE WE MEASURING?

Ana Rodriguez-Mateos[1]

[1] *King's College London, Nutritional Sciences, London, United Kingdom*

Measuring (poly)phenol intake accurately is challenging for several reasons including the lack of accurate data on food content, lack of validated assessment methods, and high variability in the (poly)phenol content of foods. Dietary assessment methods such as Food Frequency Questionnaires (FFQs) and food records have been widely used to estimate dietary (poly)phenol intake. However, they have many limitations inherent to their self-reporting nature, while biomarkers provide a more objective approach. Our research group has conducted a number of studies with the aim of comparing different methods for the estimation of (poly)phenol intake, including FFQ, 7-day food diaries, as well as plasma and urinary biomarkers. Our results showed that the agreement between FFQ and 7DD was moderate but the agreements with biomarkers measured in plasma and urine were poor. We have also developed novel tools to improve the estimation of (poly)phenol intake and adherence to (poly)phenol rich diets, such as a novel a priory (poly)phenol-rich diet score (PPS) to measure adherence to (poly)phenol-rich dietary patterns. The PPS was not only positively associated with the estimated intakes of most of the classes and subclasses of (poly)phenols from FFQ but also with multiple urinary (poly)phenol metabolites, which suggests that PPS may be a good reflection of (poly)phenol intake and exposure levels, and can be used to identify participants with higher compliance to a diet rich in (poly)phenols in a free-living population. In terms of biomarkers of (poly)phenol intake, a recent systematic review conducted by our group suggests that only very few proposed biomarkers have been validated and fulfil the criteria proposed by the FOODBALL consortium. Strengths and limitations of using biomarkers and dietary assessment tools will be discussed.

O 009 MODULATION OF THE GUT MICROBIOTA AND IMMUNE SYSTEM BY DIET

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In humans, most epithelial surfaces are colonized by a complex ecosystem of microorganisms called the microbiota. While each of these surfaces has a unique microbial colony, host-microbe interactions are necessary for our adaptation to the environment, the development of many physiological processes, and their regulation. The largest microbial community resides in the gut and disruption of the gut microbiota, i.e. dysbiosis, is associated with numerous inflammatory, metabolic, cardiovascular, and neoplastic diseases. This extraordinarily broad spectrum of effects is caused either directly by the production of bioactive metabolites or indirectly by affecting the cellular and humoral response in host cells-mainly in the epithelial and immune cells of the gut, which can even reach distant organs and tissues. Dysbiosis is often associated with impaired barrier function ("leaky gut"), leading to increased interactions of microbial antigens with the immune system and local and systemic inflammation. Therefore, the health benefits attributed to key modifiers of the gut microbiota, such as altered diet, may be mediated through the gut microbiota. Here, I will briefly describe the mechanisms of how dietary components influence immune system reactivity and outline concepts that bridge the microbiota and immune mechanisms.

O 010 NEW INSIGHTS INTO THE MICROBIOME-COCOA FLAVONOIDS INTERACTION IN AN *EX VIVO* COLONIC MODEL

Sara Dobani[1], *Jose Muñoz-Redondo*[2], *Gema Pereira*[2], *Jose Moreno-Rojas*[2], *Francesca Fava*[3], *Letizia Bresciani*[4], *Kirsty Pourshahidi*[1], *Nigel Ternan*[1], *Pedro Mena*[4], *Tahani Al Mutairi*[5], *Alan Crozier*[5], *Daniele Del Rio*[4], *Kieran Tuohy*[6], *Chris Gill*[1]

1 *Ulster University, School of Biomedical Sciences, Coleraine, United Kingdom*
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6 *University of Leeds, Faculty of Environment, Leeds, United Kingdom*

Background and Objectives:

Cocoa (*Theobroma cacao* L.) is a rich source of procyanidins and flavan-3-ols, which are bioactive compounds that reach the distal section of the upper gastrointestinal tract (GIT) mainly unabsorbed or as phase II metabolites. Investigation of the colonic catabolism of flavan-3-ols and their physiologically relevant structures represents a key aspect for understanding and exploiting their putative bioactive potential.

Methods:

Ileal fluid samples collected post consumption of a cocoa drink (containing 566 mg of flavan-3-ols) by subjects with an ileostomy (ClinicalTrial.gov registered study; NCT03765606) were characterised based upon the presence of structurally related (–)-epicatechin metabolites (SREM) and procyanidins (PC). In the current study, ileal fluid samples collected pre- and post-cocoa drink intake from five of these subjects underwent *ex vivo* faecal fermentation. Faecal samples from four different donors were individually inoculated with ileal fluid samples and incubated for 24 h using an anaerobic, temperature and pH controlled *in vitro* gut model. The resulting ileal fluid fermentates were sampled at 0, 5, 10, & 24 h, and metabolome and microbiome changes were subsequently analysed.

Results:

5-(3',4'-Dihydroxyphenyl)-γ-valerolactone, 5-(3'-hydroxyphenyl)-γ-valerolactone, 4-hydroxy-5-(4'-hydroxyphenyl)-valeric acid, and 5-(dihydroxyphenyl)-valeric acid were the main products to accumulate during the 24 h period together with smaller quantities of 5-(hydroxyphenyl)-γ-valerolactone-sulphates, most likely catabolites of sulphated SREMs and procyanidins. The different faecal donors used influenced the metabolic profile (26% total variability) and microbiome β-diversity ($R^2 \geq 0.41$, $P < 0.001$) of ileal fluid fermentates. Nonetheless, changes in microbiota composition were evident when comparing pre-cocoa and post cocoa ileal fluid fermentates. These changes were characterised by higher relative abundance in Proteobacteria at 5 h and 10 h ($P < 0.05$) and significantly higher Bacteroidetes and reduced *Clostridia* and *Erysipelotrichia* at 24 h. The Firmicutes:Bacteroidetes ratio was also generally lower in post-cocoa ileal fluid fermentates compared to controls at 10 h and 24 h. Finally, significant ($P < 0.05$) inverse correlations identified between sulphated SREMs and clades involved in gut microbial sulphidogenesis including *Desulfovibrio* were, together with other significant microbe-metabolite interactions associated with γ-valerolactones.

Conclusions:

To summarise, 24-h ex *vivo* faecal fermentation of cocoa (poly)phenols enriched ileal fluid significatively affected the (poly)phenolic and microbiome composition, with inter-individual and time-dependent based effects evident.

O 011 NATURAL PRODUCT PHYTOCHEMICAL ANALYSIS USING A COMBINED FINGERPRINTING AND CHEMOMETRIC APPROACH

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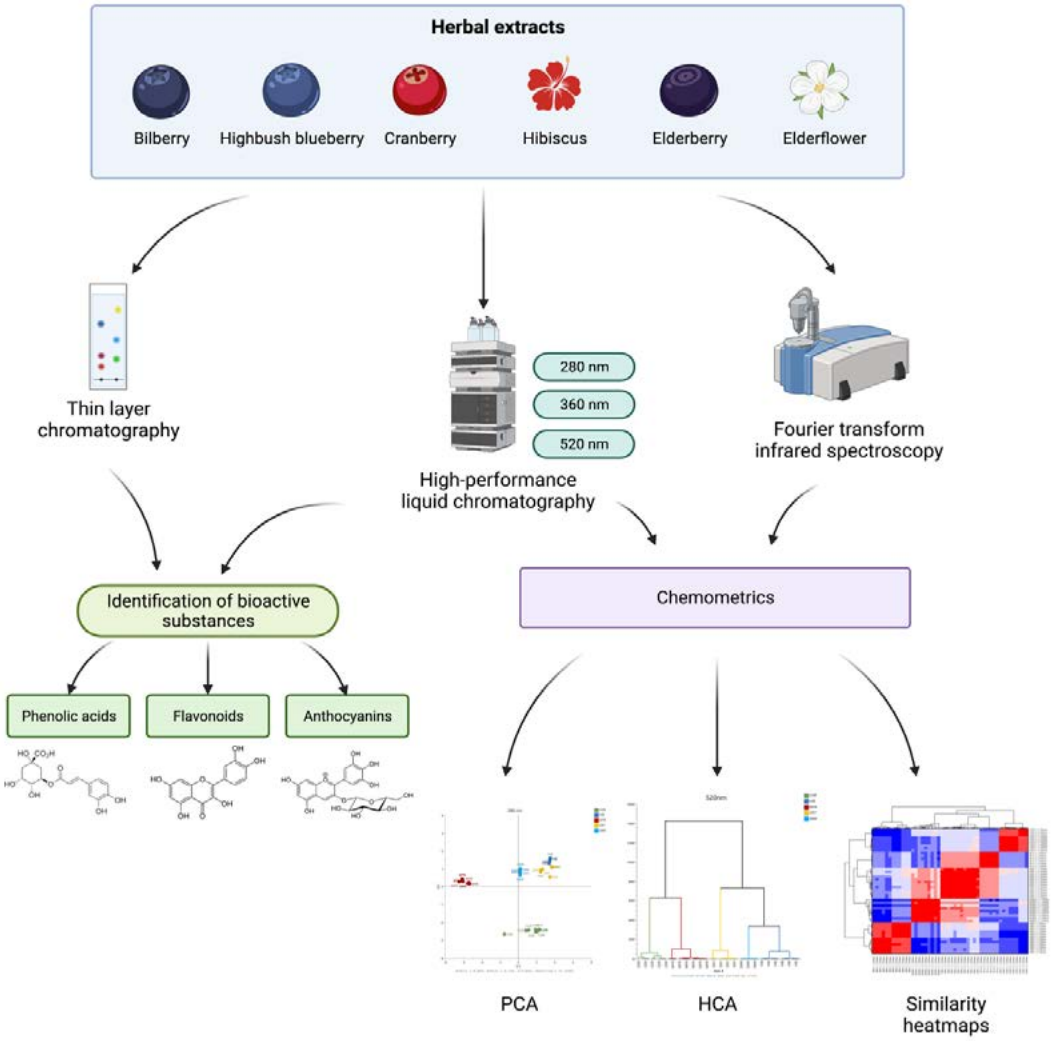
Natural products derived from medicinal plants are still used worldwide by both developing and developed countries as a therapeutic and prophylactic option to increase one's health. Traditional use is now backed with state-of-the-art research on the biological activity of plant secondary metabolites. With the increase in demand and globalization tendencies, also international trade of plants or their parts has risen exponentially. With that, the question of methods used for identifying and characterizing these materials has become increasingly pressing[1]. Herbal fingerprints provide a comprehensive chemical profile that can be used to both identify and characterize the composition of medicinal plants. The fingerprints can be obtained using various chromatographic or spectroscopic methods. To further interpret the fingerprinting data, various chemometric approaches can be applied, including principal component analysis (PCA), hierarchical cluster analysis (HCA), and others[2].

The aim of this study was to evaluate the practical application of the combined approach and to compare the advantages and drawbacks of chromatographic and spectroscopic methods. Chromatographic profiles at 3 different wavelengths (280 nm, 360 nm, 520 nm) were analyzed in more detail.

To achieve the set aims, ethanolic extracts of 6 commercially available crude drugs (hibiscus calyxes, bilberries, highbush blueberries, cranberries, elderberries, elderflowers) were made. At least 4 different samples from each crude drug were analyzed. A thin-layer chromatography (TLC) method was used as an initial screening tool. A high-performance liquid chromatography (HPLC-UV) method was developed and validated. The standard adding method was carried out to identify individual polyphenols. An ATR-FTIR method was applied to obtain spectroscopic fingerprints. PCA, HCA, similarity analysis, retention time adjustment, and heatmap visualization were carried out on Spectragryph 1.2.15., SpecAlign 2.4.1, Origin 10, and SIMCA 17 software.

Overview of the methods used in the study.



The chemical composition was determined in the scope of phenolic acids, flavonoids, and anthocyanins. All analyzed extracts, except elderflowers, contained significant amounts of anthocyanins, therefore, explaining the visual similarity between them. The similarity of samples was also visualized by Pearson's correlation coefficient heatmaps. While at 280 nm the chemical composition was the most diverse, similarity between all samples increased at 520 nm. Although quantitative differences between highbush blueberry and bilberry anthocyanin profiles were observed, the qualitative pattern of the fingerprint was almost identical. This observation was confirmed by the PCA analysis with clusters of these two fruit extracts being located close and hindering optimal separation. The HCA results revealed a correlation between the phylogenetic tree of analyzed samples and their chemical composition. The main drawback of using HPLC is retention time shifting over time. To overcome this obstacle retention time adjustment to the average chromatogram was executed thus significantly increasing the similarity between samples from one plant. Spectroscopic regions 800–1800 cm^{-1} and 2800–3200 cm^{-1} can be used to obtain fingerprints and compare them between different species. FTIR analysis showed closely related fingerprints of different parts of one plant – elderberries and elderflowers.

Phytochemical analysis using a combined fingerprinting and chemometric approach allows us to make concrete conclusions about the identity and quality of natural products.

1 https://doi.org/10.1016/j.aca.2013.09.017.
2 https://doi.org/10.1016/j.jpba.2020.113215.

O 012 BLOOD-BRAIN BARRIER TRANSPORT OF BIOACTIVES FROM MEDITERRANEAN FOODS

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Diet-based therapies have been identified as a promising approach to prevent and delay the progression of neurodegenerative diseases. In this regard, adherence to the Mediterranean diet (MD) is associated with a lower risk of dementia. Bioactive compounds such as tyrosol (TYRS), hydroxytyrosol (HT) and protocatechuic acid (PCA) are present in MD. HT and TYRS are phenols present in olives, virgin olive oil (VOO), and wine. PCA is the major phenolic acid metabolite formed from anthocyanins. Likewise, serotonin (SER) is synthesized from L-tryptophan. TYRS, HT, PCA and SER possess neuroprotective properties against Parkinson and Alzheimer disease. However, little is known about their capacity to cross the blood-brain barrier (BBB) required to reach the biological targets. Therefore, this work evaluates the percentage to pass through the BBB of tyrosine (TYR) (10 and 100 μM), HT (1 and 100 μM), TYRS (20 and 2000 μM), SER (20 and 200 μM) and PCA (1 and 100 μM) and how their structure affects the brain integrity. Human brain microvascular endothelial cells (HBMEC) are considered the most suitable human cell line for an *in vitro* BBB model concerning barrier tightness and a transwell-based system. Toxicity and BBB integrity tests in the HBMEC were performed using transendothelial electrical resistance (TEER), paracellular permeability using sodium fluorescein and immunocytochemical assays with the junctional protein integrity β-catenin. The exposition of the BBB endothelium to the bioactive compounds does not cause any change in the BBB integrity. Furthermore, the degree of transport across the BBB was evaluated by means of ultra-high-performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS/MS). Our results revealed that all the bioactives were quantifed in the upper and lower compartments at different concentrations, suggesting a differential transport of the metabolites. HT was the bioactive compound which crossed the BBB in a highest extent (70%), compared with TYR (50%), TYRS (30%), SER (30%) and PCA (9%) at the highest concentrations tested.

O 013 MODULATORY EFFECT OF EXTRA VIRGIN OLIVE OIL POLYPHENOLS AND THEIR METABOLITES ON INFLAMMATORY RESPONSE IN INTESTINAL CACO-2 AND ENDOTHELIAL HUVEC CELLS

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Background:

Extra virgin olive oil (EVOO), the main lipid source in the Mediterranean diet, is rich in bioactive compounds such as hydroxytyrosol (HT) and tyrosol (Tyr), that have been shown to possess antimicrobial, antioxidant and anti-inflammatory properties. It is well known that free forms of phenolic compounds ingested with food are largely metabolized in the intestine and liver and their concentrations after ingestion are too low to explain the biological effects observed in *in vivo* and *in vitro* models. In particular, HT and Tyr usually undergo glucuronidation and sulfonation, and their sulphated and glucuronidated metabolites are the prevalent forms found in human plasma, urine and in the gut, where they reach relevant concentrations compatible with biological activity.

Objectives:

In this context, the aim of this study was to evaluate HT and Tyr and their sulphated and glucuronidated metabolites on the inflammatory response at intestinal and endothelial level, using pro-inflammatory stimuli, such as LPS and/or a hyperglycemia (HG) condition. Their modulatory action was evaluated focusing on the alteration of tight junctions (TJ) proteins and the activation of cellular pathways, as mitogen-activated protein kinases (MAPKs) and NLRP3 inflammasome, which are linked to chronic inflammatory diseases.

Methods:

The alteration of epithelial/endothelial barrier in Caco-2 and HUVEC cells monolayer, treated with LPS or HG alone or together with EVOO phenolic compounds and their metabolites, was evaluated through cell permeability tests (TEER, FITC-Dextran permeability assay) and through determination of the disruption and/or relocation of TJ proteins, in relation to redox-sensitive MAPKs modulation and activation of the NLRP3 inflammasome.

Results:

Obtained data showed that HG and physiopatologically relevant concentration of LPS increase cellular membrane permeability in both Caco-2 and HUVEC monolayers, through the alteration of occludin, zonulin and JAM-A TJ proteins, following the activation of pathways involved in the inflammatory process such as p38 and ERK 1/2 MAPKs and NLRP3 inflammasome. Contextually, the pretreatment with physiologically concentration of HT, Tyr and their sulphated and glucuronidated derivatives induced a protective effect, limiting the alteration of TJ proteins and the activation of MAPKs and NLRP3 inflammasome, strengthening the hypothesis that HT and Tyr, as well as their metabolites, may exert a significant role in the maintenance of intestinal and endothelial barrier integrity. Finally, it was observed that HT and Tyr metabolites and their parent compounds exert a comparable efficacy both at intestinal and endothelial level, interacting with intracellular signaling involved in the modulation of cellular pro-inflammatory response.

Conclusion:

All these findings suggest that EVOO-derived phenolics parental free forms and their major *in vivo* formed metabolites, which represent the largest part of a continually changing pool of compounds, are responsible, as a whole, for the observed beneficial effects in the prevention and amelioration of the major intestinal and cardiovascular degenerative diseases.

O 014 CHRONIC AND POSTPRANDIAL EFFECT OF BLUEBERRIES ON COGNITIVE FUNCTION, ALERTNESS AND MOOD IN PARTICIPANTS WITH METABOLIC SYNDROME – RESULTS FROM A 6-MONTH, DOUBLE BLIND, RANDOMIZED CONTROLLED TRIAL

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Background:

Population based studies suggest that a higher habitual intake of anthocyanins and blueberries are associated with improved cognitive function. However, to date, the randomized controlled trial (RCTs) data are mixed – with greater effects shown in those with pre-existing cognitive dysfunction or experiencing increased cognitive loads. To date, the effect of acute or chronic blueberry intake in adults with metabolic syndrome, but without cognitive dysfunction, have not been established.

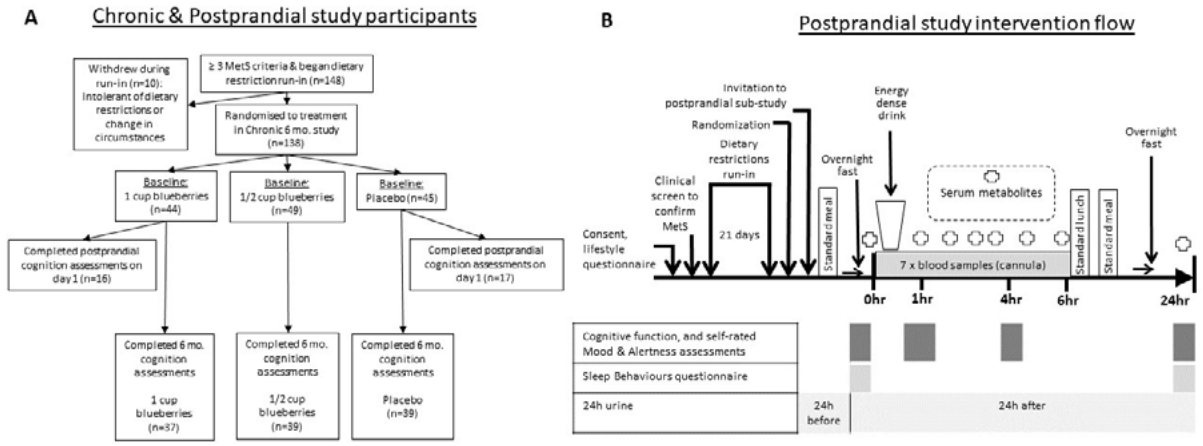
Objective:

Cognitive function, mood and alertness, and sleep quality were assessed acutely (over 24 h) following a postprandial challenge and in a 6-mo blueberry intervention trial (0.5 and 1 cup a day), in those with metabolic syndrome (MetS).

Methods:

A double-blind, parallel RCT (n=115; age 63±7 years; 68% male; BMI 31.2±3.0 kg/m^2) was conducted, with freeze-dried blueberry intakes (equivalent to ½ and 1 cup/d) compared with matched placebo. In a sub-set, a postprandial assessment was also made, comparing 1 cup/d blueberry *versus* isocaloric matched placebo, each provided within an energy-dense drink (969 Kcals, 64.5 g fat, 84.5 g carbohydrate, 17.9 g protein). Mood and alertness, and cognitive function, were assessed respectively, using i) Bond Lader self-rated scores, and ii) an electronic test battery of 10 assessments (CDR test battery), based on the composite cognitive function domains of *attention*, *working memory*, *episodic memory*, *working & episodic memory*, *speed of retrieval from memory*, *executive function*, and *picture recognition*. The impact of sleep status was evaluated using the Leeds Sleep Evaluation Questionnaire. Anthocyanin metabolism was assessed (urinary and serum), and ApoE status determined to account for potential impacts on cognition considered *a priori*. Intervention foods and study funding was provided by the U.S. Highbush Blueberry Council.

Figure 1: Participant recruitment & retention, and postprandial study flow



Results:

6-mo daily blueberry intake did not affect mood, sleep or cognitive function in adults with MetS ($p>0.05$); with only improved picture recognition accuracy ($p=0.06$) and self-related alertness ($p=0.07$) approaching statistical significance. A lack of postprandial benefit was also observed, with the exception of a significant improvement in self-rated *contentment* after 1 cup blueberry intake ($p=0.01$). In exploratory metabolite analysis (across both the ½ and 1 cup/d intakes), an increase in catechin and chlorogenic acid derivatives (i.e. *hydroxycinnamic acids*, *benzoic acids*, *phenylalanine derivatives* and *hippuric acids*) were associated with favourable chronic and postprandial cognitive function ($p\leq0.05$). Most notably, memory (i.e. *speed*, *working*, *episodic*, and *picture recognition*), attention (i.e. *continuity*, *power*, *alertness*, and *wakefulness*), as well as *executive function,* self-rated *contentment* and *quality of sleep*.

Conclusions:

Whilst we observed an acute improvement in self-rated contentment and identified *cognition-metabolite* associations ($p\leq0.05$) for future hypothesis generation, overall our data do not support an improvement in cognitive function, alertness, mood or sleep quality in participants with MetS. Consequently, our data reinforce the increasing evidence that cognitive benefits of blueberries are more likely to be experienced in those with pre-existing cognitive dysfunction and may be less effective in those with MetS and at elevated CVD risk.

O 015 PROANTHOCYANIDINS MODULATE CENTRAL CLOCK, RESTORE DIURNAL RHYTHMICITY, AND IMPROVE CARDIOMETABOLIC RISK FACTORS IN CAF DIET-INDUCED OBESE RATS IN A TIME-OF-DAY-DEPENDENT MANNER

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Circadian rhythms play an important role in the physiological and metabolic adaptation of the organism and their disruption increase the metabolic risk. Proanthocyanidins (PACs) are phenolic compounds which have demonstrated beneficial properties on metabolic disorders, although the bioactivity of these compounds may vary depending on the moment of their administration. Different molecular mechanisms are involved in their healthy properties, including modulation of the circadian system. Therefore, the aim of this study was to evaluate whether the time of PACs administration can modulate their effects in an obesogenic context and if these phenolic compounds can restore the circadian disruption caused by a calorie-dense diet. To do so, sixty-four Fisher rats were fed standard or cafeteria diet (CAF) for 5 weeks. After this, animals were administered a daily dose of 25 mg/kg of a grape seed proanthocyanidin-rich extract (GSPE) or vehicle (VH) either at 8 a.m. or 8 p.m., (ZT0 and ZT12, respectively) for 4 weeks. Animals were sacrificed at different times, including ZT1, ZT7, ZT13 and ZT19. Results showed the detrimental effects of CAF diet on diurnal rhythmicity of serum biochemical parameters and hormones and hypothalamic clock genes. GSPE administration improve the metabolic health of animals and restored the oscillations of their biochemical parameters, hormones and clock and appetite signaling genes in a time-of-day-dependent manner, showing more effects when administered ZT12. Notably melatonin rhythmicity, a key marker of the light/dark cycle, was restored by GSPE treatment at ZT12. In conclusion, PAC administration improved metabolic status in CAF-fed rats and restored dirunal oscillation of central clock. Although further investigations are needed to elucidate the specific effect of PACs in these conditions, these results suggest that these phenolic compounds may modulate the diurnal rhythmicity of the central clock contributing to the improvement of the metabolic profile, especially, when PACs are administered at night.

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O 016 FROM DIET TO NEURONAL CELLS: INVESTIGATING THE EFFECTS OF LOW-MOLECULAR WEIGHT (POLY)PHENOL METABOLITES ON PARKINSON'S DISEASE

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Background:

Parkinson's disease (PD) is a progressive neurodegenerative disorder mainly affecting dopaminergic neurons from the nigrostriatal pathway. Currently, the main challenges focus on its progressive nature and its limited treatment options for symptom management. Extensive research on neurodegenerative disorders has identified (poly)phenols as potential beneficial agents for brain health. However, the brain accessibility and PD-related benefits of low-molecular weight (poly)phenol metabolites, which are highly abundant in the bloodstream resulting from the colonic metabolism of dietary (poly)phenols, are still not fully understood.

Objectives:

This study aimed to investigate the potential of BBB-permeant dietary (poly)phenol metabolites to protect the brain by using a 3D cellular model of PD and how a berries-enriched is capable of preventing PD-like symptoms *in vivo* by using an MPTP mouse model.

Methods and Results:

In vitro experiments with human brain endothelial cells in transwell systems revealed the transport kinetics, molecular mechanisms of BBB metabolism, and brain uptake pathways of in-house synthesized metabolites. *In vivo* experiments with male Wistar rats showed that these low-molecular weight injected (poly)phenol metabolites could rapidly cross the BBB and reach the brain. Moreover, (poly) phenol metabolites displayed distinct abilities to protect dopaminergic neurons on a 3D cell model of PD by modulating glutathione metabolism, heat-shock response, and pro/anti-apoptotic proteins through preconditioning effects. Berries supplementation significantly improved motor strength and coordination in MPTP mice and delayed the onset of neurodegeneration markers, such as Tyrosine Hydroxylase. Moreover, berries enriched-diet prevented the increase of microglial reactivity in Substantia Nigra pars compacta, striatum, and motor cortex.

Conclusion:

Overall, the findings suggest that circulating (poly)phenol metabolites have the potential to reach the brain and tackle some important features related to neurodegeneration found in PD.

O 017 A MILK PROTEIN HYDROLYSATE DECREASES INFLAMMATION ON A MODEL OF mBSA-INDUCED ARTHRITIS MICE

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Objectives:

Osteoarthritis affects over 500 million people worldwide. Due to the global aging of the population, this incidence is constantly increasing.

In order to relieve pain, people need to take non-steroidal anti-inflammatory drug (NSAI), however NSAI have known for their side effect. Thus, a natural solution, without side effects, to relieve inflammatory and pain in case of joint issue would be very interesting.

In this way, we evaluated the efficiency of a milk protein hydrolysate in an *in vitro* model of inflammation and in an arthritis model of mice.

Methods:

In vitro experiment have been performed to evaluate casein hydrolysate on cytokine production (TNFα, IL1β, IL10) by Peripheral Blood Mononuclear Cells (PBMC) stimulated by LPS. Five doses of the casein hydrolysate have been tested and compared to a drug positive control, dexamethasone.

After which an *in vivo* experiment has been performed to validate *in vitro* data. Casein hydrolysate has been evaluated in a model of rheumatoid arthritis induced by methylated bovine serum albumin (mBSA) in C57BL/6J mice, inducing the formation of an oedema in knee joints. For 29 days, mice received orally drinking water or casein hydrolysate at different concentration: 10, 50 and 250 mg/kg.

Results:

The lowest dose of casein hydrolysate inhibit production of TNFα, IL1β, and IL10. Its effect is similar to the anti-inflammatory effect of dexamethasone.

In vivo study has shown that after oedema formation due to the mBSA, mice receiving the casein hydrolysate at 50 mg/kg and 250 mg/kg have a significant reduction of the oedema compared to the mBSA group treated with the vehicle.

Conclusions:

These *in vitro* and *in vivo* experiments demonstrated that casein hydrolysate have anti-inflammatory properties allowing to decrease by more than 60% the size of the knee oedema in mice suffering from arthritis compared to the vehicle mice. These results are promising and would make it possible to have a natural product to replace, at least in part, the taking of chronic anti-inflammatory drugs. The next step is to validate these results with a clinical study.

O 018 MEMBRANE FILTRATION PROCESSING OF INFANT MILK FORMULA PROMOTES GUT MATURITY *IN VITRO* AND *IN VIVO*

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Background:

We recently produced 250 kg of infant milk formula (IMF) powder at pilot scale using membrane filtration (MEM-IMF) and high temperature (HT-IMF) processing. Both IMFs had the same ingredients and were spray dried. MEM-IMF had significantly higher content of native whey protein (59.9%) compared to HT-IMF (4.5%) ($P < 0.001$). This resulted in significantly different protein digestion kinetics.

Objectives:

The objective of this study was to investigate, *in vitro* and *in vivo*, the impact of IMF processing on gut barrier physiology.

Methods:

For *in vivo* studies 20 Large White × Landrace pigs (28 days old) were randomly assigned to two treatment diets (**1**) MEM-IMF or (**2**) HT-IMF where IMF accounted for 35% of their diet. Pigs were fed twice daily for 28 days and slaughtered 3 hours after their final feeding. Gut lumen and tissue samples were taken immediately from three locations of the small intestine (duodenum, jejunum and ileum). For *in vitro* studies, IMF powders were subjected to *in vitro* static gastrointestinal digestion using the INFOGEST protocol adapted to infant conditions. Polarised 21-day old Caco-2:HT29-MTX (90:10) monolayers, representing the small intestinal gut barrier, were treated for four hours with *in vitro* digested MEM-IMF or HT-IMF or pig gut lumen samples.

Results:

MEM-IMF fed pigs had a significantly higher number of goblet cells in the jejunum (17.39 ± 1.43) compared to piglets fed HT-IMF diet (11.7 ± 1.27) ($P < 0.05$). Acidic mucins were significantly increased in the jejunum of pigs fed MEM-IMF compared to those fed HT-IMF ($P < 0.05$). In addition, protein levels of MUC-2 were significantly increased in jejunal mucosal scrapings in pigs fed MEM-IMF compared with HT-IMF fed pigs ($P < 0.05$). However, processing type had no significant effect on villus height or crypt depth in either the duodenum or jejunum. Treatment of polarised co-culture monolayers with MEM-IMF *in vitro* digesta significantly up-regulated mRNA transcript levels of mucin biomarkers (*MUC-1* and *MUC*-2) and the tight junction gene *OCLN* compared to the HT-IMF digesta ($P < 0.05$). Furthermore, when polarised Caco-2:HT29-MTX monolayers were treated with *in vivo* pig jejunum digesta, mRNA transcript levels of the mucin gene, *MUC-2,* and the tight junction genes, *CLDN-1* and *OCLN,* were significantly increased in the MEM-IMF group compared to the HT-IMF group ($P < 0.05$).

Conclusion:

The findings of this study suggest that producing IMF by membrane filtration not only produced a product, which is easier to digest, but also promoted gut maturity.

O 019 OAT PROTEIN MODULATES CHOLESTEROL LEVELS AND IMPROVES CARDIAC SYSTOLIC FUNCTION IN HIGH-FAT, HIGH-SUCROSE FED RATS

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Oats are recognized to provide many health benefits that are mainly associated with its dietary fibre, β-glucan. However, the protein derived from oats is largely understudied with respect to its ability to maintain health and attenuate risk factors of chronic diseases. The goal of the current study was to investigate the metabolic effects of oat protein consumption in lieu of casein as the protein source in high fat, high sucrose (HF/HS) fed Wistar rats. Four week old rats were divided into three groups and were fed three different experimental diets: a control diet with casein as the protein source, a HF/HS diet with casein or a HF/HS diet with oat protein for 16 weeks. Heart structure and function were determined by echocardiography. Blood pressure measurements, an oral glucose tolerance test, and markers of cholesterol metabolism, oxidative stress, inflammation and liver and kidney damage were also performed. Our study results show that incorporation of oat protein in the diet was effective in preserving systolic heart function in HF/HS fed rats. In addition, oat protein lowered serum total- and LDL-cholesterol. To conclude, oat protein provides hypocholesterolemic and cardioprotective benefits in a diet induced model of metabolic syndrome.

O 020 BIOACPEPFINDER: NOVEL BIOINFORMATICS TOOL FOR THE DISCOVERY OF BIOACTIVE PEPTIDES FROM PROTEIN DIGESTION

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For several decade, health and alternative medicine sectors focus their research on the bioactive peptides (BAPs) derived from food, due to their preventive and/or health benefit applications. However, the experimental approaches for BAP discovery remain long and challenging. As a consequence, *in silico* methods proved to be more strategic due to the time and cost savings, and their efficient prediction of potential BAPs [1].

In this context, we developed a comprehensive pipeline, called BioacPepFinder, that is able to perform large- scale screening in order to discover new BAPs from *in silico* enzymatic hydrolysis of proteins. This pipeline takes as input a set of amino acid sequence of proteins (in FASTA format or with Ensembl, Uniprot or NCBI id). The first step is to simulate *in silico* hydrolysis, with RPG [2] coupled to an in-house script to deal with missed cleavages. The list of generated peptides is then filtered according to two complementary criteria. Peptides are compared with Blast to specialized database of BAPs such as BIOPEP [3] and DRAMP [4], that contain known peptide sequences displaying proven bioactivities. We also compute for each peptide the quantitative structure-activity relationship (QSAR) score for discovering novel and potent BAPs [5]. This score is based on crucial structural properties, specific to peptides with angiotensin-converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV) inhibitory activities.

BioacPepFinder is implemented in Python and dependencies are managed with Conda. This bioinformatic tool is modular, as each step can be carried out separately and also extensible: new proteases (for the hydrolysis), new databases or new criteria to select peptides (with wrappers) could be added. This software is freely accessible at https://gitlab.univ-lille.fr/bilille/bioacpepfinder.

BioacPepFinder was evaluated for its capability to predictBAP by performing *in silico* tests on standard peptides and proteins such as bovine hemoglobin or serum albumin. In this context, BioacPepfinder was used to predict bioactivities of proteins of agri-food origin while *in vitro* bioactivity tests were performed concomitantly. Results proved that this tool would be a new high-efficient prediction software that aims for guiding development and optimization of BAPs.

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O 021 EFFECTS OF INDIVIDUAL AMINO ACIDS ON APOA-I MRNA PRODUCTION AND PRO-APOA-I SECRETION

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Background:

Apolipoprotein A-I (ApoA-I), the major protein of high density lipoprotein (HDL) particles, plays a crucial role in reverse cholesterol transport (RCT). A higher concentration of ApoA-I is associated with both an increased HDL functionality and RCT. A promising strategy to prevent cardiovascular diseases can be to improve RCT by increasing *de novo* ApoA-I production. While some dietary components have shown to affect HDL functionality, effects of dietary proteins or individual amino acids have not been considered to the same extend. Although animal studies have shown a possible association between amino acid intake and hepatic lipid metabolism, the exact effects of individual amino acids on hepatic ApoA-I production and underlying mechanisms remain unclear. Therefore, we here examined the effects of different amino acids on hepatic ApoA-I mRNA transcription and pro-ApoA-I secretion as experimental animal models have indicated that amino acids effects the hepatic lipoprotein metabolism.

Methods:

Human hepatocytes (HepG2) were exposed to either a BET inhibitor (JQ (+)) as positive control for ApoA-I transcription or different dosages (0, 2, 5 or 10 mM) of amino acids (glutamine, glutamic acid, histidine, leucine, proline or tryptophan) for 48 hours. ApoA-I mRNA expression and pro-ApoA-I protein secretions were analyzed using quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assays (ELISA), respectively. To study the underlying mechanisms we analyzed CPT1 and KEAP1 mRNA expression with qPCR (withTaqMan Gene Expression Assays). The peroxisome proliferator-activated receptor alpha (PPARα) transactivation was measured via pcDNA3.1 co-transfected cells including a PPARα response element cloned in front of a reporter gene luciferase. Therefore, the luciferase activity could be measured which reflects the PPARα transactivation. Finally, the mechanistic target of rapamycin complex 1 (mTORC1) phosphorylation was measured via a western blot. Regression analyses were used to examine dose-response relationships between the concentrations of added amino acids. For the correlations between CPT1 mRNA expression and ApoA-I mRNA expression, Spearman correlations were calculated. The effects of individual doses were statistically evaluated versus control conditions by Mann–Whitney U tests. In all used statistical tests a p-value < 0.05 was considered to be statistically significant.

Results:

JQ (+) increased the ApoA-I mRNA transcription and pro-ApoA-I protein secretion in HepG2 cells, indicating that the cells were responsive. A significant dose-dependent increase in ApoA-I and CPT1 mRNA expression was seen in the leucine, glutamic acid and tryptophan treatment, with the largest increase in the 10 mM treatment of these amino acids (32%, 21% and 47% respectively). Additionally, tryptophan (10 mM) strongly increased PPARα transactivation (>7-fold increase vs control). Glutamine, proline, and histidine significantly increased pro-ApoA-I protein concentrations in a dose-dependent manner. mTORC1 phosphorylation remained unchanged after amino acid treatment in the HepG2 cells.

Conclusion:

Individual amino acids have different dose-dependent effects on ApoA-I mRNA expression and pro-ApoA-I protein secretion. These effects are in line with specific effects on PPARα transactivation and activity. This indicates a clear role for PPARα activation as potential mechanism while there is no indication for involvement of the mTORC1 pathway.

O 022 BIOACTIVE PEPTIDES FROM MILK CONSUMPTION: POSTPRANDIAL CIRCULATING PROFILES AND IMPACT ON GASTROINTESTINAL DISCOMFORTS IN HEALTHY SUBJECTS

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Background:

Cow milk-related gastrointestinal discomforts (GID) are steadily increasing among healthy and lactose intolerant individuals. The cause of milk-induced GID in humans is still a topic of scientific debate, with some bioactive peptides (BAPs) derived from the proteolysis of β-casein being a focus of research. However, there is conflicting evidence and a lack of studies demonstrating a clear causal relationship between milk protein digestion and GID in healthy individuals.

Objectives:

This study aimed to investigate milk protein digestion in two groups of participants: 19 participants with milk-related GID and nonhabitual milk consumers [NHMCs] and 20 participants without GID and habitual milk consumers [HMCs].

Methods:

Overnight fasting participants consumed 250 mL of milk, after which blood was collected over the next 6 hours to evaluate the postprandial responses of 31 milk-derived bioactive peptides (BAPs), 20 amino acids, and four hormones. The participants also reported any GID experienced over 24 hours after milk intake.

Results:

In HMCs circulating BAPs peaked at 30 min upon milk consumption and showed a higher area under the curve over 6 hours compared to NHMCs. Indeed, in NHMCs the circulating BAPs weakly peaked only at 4 h after milk consumption. Looking at BAPs bioactivities, NHMCs showed a higher circulating opioid agonists-to-antagonists ratio over the first hour compared to HMCs; this could have slowed gastrointestinal transit affecting GID. The plasma amino acids concentrations were similar in the two groups. Results were independent of small and large intestinal gut permeability.

Conclusion:

Altogether, the results showed that an impaired milk protein digestion along with a different circulating level of opioid agonists-to-antagonists BAPs could affect milk-induced GID in healthy and lactose-tolerant individuals.

O 023 APOE GENOTYPE, THE GUT MICROBIOTA AND POLYPHENOL METABOLISM INTERACTIONS

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The *APOE* genotype is a significant genetic risk factor for Alzheimer's disease, affecting 40–60% of patients who carry at least one *APOE4* allele. Recent evidence shows that this genotype could also impact the gut microbiome's structure and function, in addition to its effects on ageing and the brain. Dietary polyphenols, which are bioactive molecules found in fruits and vegetables, can slow cognitive decline and affect gut microbiota speciation and metabolism. However, there hasn't been any exploration of the differences in the metabolism of these compounds according to *APOE*genotypes. To address this knowledge gap, we conducted an animal study *in APOE3* and *APOE4* targeted replacement (TR) mice. The animals were fed a low-fat diet (10 kcal% from fat), a high-fat diet (45 kcal% from fat), or a high-fat diet supplemented with flavan-3-ols rich cocoa extract (100 mg/Kg body weight) for 16 weeks. This model has been widely used as a physiologically relevant model for age-related neuroinflammation and cognitive decline in humans, with consistently higher levels in the *APOE4*-TR vs *APOE3*-TR mice, particularly if fed a "western type" high-fat diet. Significant shifts in microbiome beta diversity were observed in *APOE3-TR* mice only under a high-fat diet, with these mice acquiring an *APOE4*-TR-like phenotype. The addition of flavan-3-ol rich cocoa reversed this shift by increasing *rc4_4*, *Prevotella*, *Aldercreutzia* and *Faecalibacterium* genera (Log LDA score >2; FDR p adjusted 0.1) in *APOE3*-TR mice. Surprisingly, flavan-3-ol rich cocoa did not affect the structure of the *APOE4* mice's microbiome. Such results were paralleled by an increased urinary excretion of flavan-3-ols colonic derived metabolites 5-(3',4'-dihydroxyohenyl)-γ-valerolactone ($p<0.05$), 5-(hydroxyphenyl)valeric acid-4-sulfate ($p<0.01$) and 4-hydroxy-5-(hydroxyohenyl)valeric acid sulfate ($p<0.01$) in *APOE4*-TR mice. These preliminary findings were further confirmed in a human cohort study (COMBAT Study; NCT03679533) following a 12-week consumption of flavan-3-ols rich cranberries along with an acute study using flavan-3-ol rich foods (COB study, NCT01922869). The relationship between polyphenols intake and *APOE*genotype is intriguing, and further work is required to gain a better understanding of the physiological and molecular mechanisms underlying such disparity.

O 024 CAN PHASE II METABOLITES OF HESPERIDIN EXPLAIN THE BENEFICIAL EFFECTS OF ORANGE FLAVANONE ON VASCULAR ENDOTHELIAL FUNCTION?

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In a previous randomized cross-over controlled trial, we have demonstrated that orange juice decreases diastolic blood pressure when regularly consumed and postprandially increases endothelium-dependent microvascular reactivity in healthy volunteers (1). In addition, our study showed that hesperidin, the major flavonoid in orange, contributes to the vascular protective effects of orange juice. In order to decipher the vascular effects associated to hesperidin, we tested the impact of their main phase II metabolites on vascular endothelial functions.

The endothelial dysfunction induced by a nutritional stress is reflected by an altered vascular reactivity, an increased release of extracellular vesicles (EVs) and changes of the expression of genes associated to endothelial activation. In the present study, these endothelial responses have been assessed either on primary aortic endothelial cells or on isolated murine mesenteric arteries exposed to a mix of hesperetin-3'-glucuronide and hesperidin-7-glucuronide and in experimental conditions mimicking a postprandial nutritional stress (e-g. a low-grade inflammation associated to a hyperglycaemia).

Among the effects of phase II metabolites of hesperidin on vascular endothelial function, this study highlights that these metabolites prevent the release of endothelial EVs induced by a nutritional stress. In addition, they reverse qualitatively and quantitatively the biological message conveyed by EV miRNAs as shown by the changes in the miRNA profile of EVs produced by activated endothelial cells exposed to the mix of hesperidin metabolites compared to the EV miRNA profile from activated cells. This result indicates that hesperidin metabolites can counteract some features associated to endothelial dysfunction. The assessment of the impact of the hesperidin phase II metabolites on the endothelial reactivity is ongoing.

Taken together, the data generated from this study will provide new insights into the mechanisms by which the main plasma phase II metabolites of hesperidin can affect endothelial function and thus support the contribution of flavanones in the beneficial effects of citrus foods on vascular function.

O 025 SPIRULINA PROTEIN-POLYPHENOL PARTICLES ATTENUATE POLLUTION-INDUCED SKIN DAMAGE: A NOVEL SUSTAINABLE APPROACH

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Pollution aggressors induce biochemical and metabolic changes that cause premature skin aging, activate inflammatory pathways and generate oxidative stress responses that ultimately affect dermal integrity and compromise overall skin health. In this project, our goal was to establish an efficient synergistic cosmeceutical route by using Spirulina protein as both carrier agent and donor of phytochemicals complexed with bioactive compounds from muscadine grape (MG) pomace to create unique, clean label-ingredients for skin health applications. The protein-polyphenol particles were used for the formulation of a topical product destined to reduce pollutant penetration, improve skin barrier function and mitigate pollution skin damage caused by diesel engine exhaust (DEE), a major particulate matter pollutant. Initially Spirulina protein-grape pomace polyphenol particles (SP-MG) were prepared by spray drying (SD; B-290, Buchi Labortechnik AG, Switzerland; inlet temperature 150 °C, outlet temperature 75–80 °C) concentrated MG pomace extract with Spirulina protein. SD efficiency was determined based on the SD solids recovery (SR). SP-MG particles phytochemical content (polyphenol load and phycocyanin content) and antioxidant activity (DPPH method) were characterized. Then, a gel was prepared with 0.25% (w/v) of xanthan gum and 100 µg/mL of spray dried SP-MG particles. The Spirulina protein-polyphenol gel or vehicle only (xanthan gum 0.25%; w/v solution) were topically applied to human skin biopsies, and after 24 h of pretreatment, biopsies were exposed for 30 min to DEE generated by a Kubota RTV-X900 diesel engine (3-cylinder, 4-cycle diesel with overhead valves, 1123 cc that has 24.8 HP at 3000 rpm) for further assessment of SP-MG's ability to mitigate pollution-induced skin inflammatory responses. An efficient SD process was established (SR 78%). Stable reddish SP-MG particles had high total phenolic content (86.83 ± 1.91 mg GAE/g sample), 3,5-diglucosyde anthocyanins and a remarkable concentration of proanthocyanidins (429.30 ± 24.00 mg PAC-B2/g sample). Phycobiliproteins (C-phycocyanin 67.70 ± 5.00 mg/g; allo-phycocyanins 38.9 ± 3.13 mg/g), carotenoids and chlorophylls a and b were present in SP-MG particles. Because of the diverse and concentrated content of phytochemicals in the particles, high antioxidant activity was also reported (DPPH 339.24 ± 1.56 µmol TE/g). The effects of SP-MG topical application on the regulation of inflammatory gene cyclooxygenase 2 (COX2) and 4-hydroxy-2-nonenal (4HNE), a cytotoxic reactive aldehyde lipid peroxidation product, were examined in human skin biopsies exposed to DEE. Results from both COX2 and 4HNE markers revealed that skin exposure to DEE caused protein damage mediated by lipid peroxidation, while topical application of SP-MG gel mitigated this effect. Topical application of SP-MG enhanced the production of Involucrin, a key protein involved in the differentiation and maintenance of proper skin barrier function, when compared to untreated human skin explants. Taken altogether, the use of natural phytochemical-rich particles from repurposed secondary waste streams and Spirulina algae protein is a cosmeceutical strategy to alleviate pollution-induced skin damage and improve dermal barrier function and integrity.

O 026 THE EFFECT OF ANTHOCYANIN RICH PLUM ON LEARNING AND INFLAMMATION: ROLE OF IL-6

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Background:

Mild Cognitive Impairment (MCI) is a condition that involves cognitive deficits beyond that of normal ageing and is often a transitional condition that precedes progression to Alzheimer's Disease (AD). Higher levels of inflammatory cytokines have been observed in MCI and AD compared to controls, including interleukin-6 (IL-6), which is associated with a risk of cognitive deterioration. Therefore, compounds that target IL-6 and cognitive function are of interest for research into novel therapeutics for MCI. Anthocyanins are naturally occurring compounds that provide the red, purple and blue pigmentation in plants and have been shown to reduce inflammation and improve cognition. However, whether anthocyanins can improve cognition and inflammation in older adults with MCI is unknown.

Objectives:

To investigate the effect of an anthocyanin-rich fruit, the Queen Garnet Plum (QGP), on learning and inflammation in older adults with MCI compared to the control (placebo), and to examine statistical correlations between these parameters.

Method:

This study is part of a larger clinical trial. Forty-two participants with MCI [20 female, 22 male, mean age 75.76±1.05 years] participated in a double-blind, randomised controlled trial (Australian New Zealand Clinical Trials Registry: ACTRN12618001184268). Participants were randomised to either the treatment group (QGP juice) or placebo (apricot juice, control) and consumed 250 mL of juice daily for 8-weeks while attending a Cognitive Rehabilitation Group (CRG) program. The Rey Auditory Verbal Learning test (RAVLT) was administered and learning rate (Trial 5 minus Trial 1; T5-T1) was calculated. In addition, levels of serum inflammatory marker IL-6 were measured before and after the nutritional intervention. Results were analysed using independent sample t-tests (one-sided) to assess whether the treatment group improved compared to the controls, and relationships were assessed using Pearson Correlations.

Results:

The QGP juice contained 144.5 mg cyanidin-3-glucoside (C3G) eq. anthocyanins/250 ml, while no anthocyanins were detected in the control (apricot) juice. There was no significant difference in learning between treatment and control at baseline ($t(40)=2.26$, $p=.107$), whereas a non-significant trend towards an improvement in learning rate for the treatment group (mean difference between T5 and T1=4.86±.51; 1.2 words per trial) compared to control (3.67±.54; 0.9 words per trial) was observed post-treatment ($t(40)=1.598$, $p=.059$). The difference in IL-6 levels was not significantly different in males ($t(18)=-1.06$, $p=.209$), whereas females in the treatment group had a significant reduction in IL-6 compared to controls ($t(17)=-1.77$, $p=.012$). There was a significant negative correlation between learning and IL-6 levels ($r=-.485$, $p=.002$) that was not apparent prior to treatment ($p=.335$).

Conclusion:

High anthocyanin nutritional interventions may be effective for improving learning in older adults with MCI. This effect may occur through the ability of anthocyanins to reduce pro-inflammatory IL-6 levels, and IL-6 correlated with learning improvements in the present study. Further research into the cognitive and anti-inflammatory benefits of a high anthocyanin dietary intervention employing a larger sample size, with sufficient power to investigate sex-specific effects is justified.

O 027 MENTAL HEALTH IN NEW PARENTS: A RANDOMISED CONTROL TRIAL INVESTIGATING DIETARY FLAVONOID INTAKE AND MENTAL HEALTH IN THE POSTPARTUM PERIOD

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Background:

During the postpartum period, parents face psychological challenges and resultantly, changes in mood and associated mood disorders such as postpartum depression (PND), have become increasingly prevalent in the 6-months following birth. Dietary flavonoids have been found to benefit mood and are therefore an appealing non-pharmacological option for potentially treating mood disorders in the post-partum. Indeed, previous research has shown a 2-week dietary intervention can reduce symptoms of anxiety and improve perceived quality of physical health during the 1-year postpartum period.

Objective:

The aim of this study was to investigate whether a two-week dietary flavonoid intervention would improve parents' mental health in the postpartum period.

Methods:

The study employed a randomised, parallel groups, controlled design to explore the effects of a flavonoid intervention versus control group on several outcomes, including mood (PANAS), postpartum depression (EPDS), postpartum anxiety (PSAS-RSF-C) and quality of life (WHOQOL). Sixty participants (mothers n=40, fathers n=20) in the 6-month post-partum period were randomised to either a 'flavonoid' or 'control' condition. The flavonoid group (n=15) were asked to add two flavonoid-rich foods (approximate flavonoid intake 218.58 mg/day) from a pre-determined list into their daily diet whilst controls (n=23) were asked to continue with their usual diet for two-weeks.

Results:

Significant effects were found in the flavonoid group where mothers reported higher positive affect and lower postpartum depression after the two-week intervention relative to baseline. This finding is especially relevant as a clinical reduction in postpartum depression scores in the flavonoid group by on average 2.6 scoring points was observed, which equated to a reduction from 'possible depression' at baseline to 'little or no depression' at 2-weeks. This change was not seen in the control group. Comparatively, fathers did not engage effectively with the intervention meaning there was not sufficient data to undertake a paternal analysis.

Conclusion:

This study provides evidence for the benefits of a dietary flavonoid intervention for mood and mental health in new mothers, supporting the utility of non-pharmacological, self—administrable changes to the diet for improving positive mood outcomes and reducing symptoms of postpartum depression in mothers during an especially challenging time. Further research for the effect of dietary interventions on paternal mental health is needed, and in particular, work is needed regarding how best to engage fathers in nutritional intervention research.

O 028 THE BATTLE OF SYNBIOTIC TREATMENTS AGAINST CELIAC DISEASE. A ROAD MAP VIEW IN DIETARY COMPOUNDS-PROBIOTIC INTERACTIONS

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Gluten-containing grains such as wheat, barley or oats are widely consumed, providing approximately 50% of the caloric intake worldwide[1]. This condition was thought to be very rare outside Europe and relatively ignored by health professionals and large population have remained undiagnosed. However, in recent years, the advances to a proper management of diagnostic and pathogenic milestones has led to the emergence of celiac disease (CD) from obscurity to global prominence[1]. These modifications have prompted experts worldwide to identify effective strategies for follow-up of CD while controlling the increase in prevalence. To date, the only effective treatment is the strict avoidance of gluten from diet but accidental ingestion of gluten proteins is almost inevitable. The use of probiotics, prebiotics or synbiotic treatments have been proposed to attenuate the intestinal barrier damage after gluten ingestion[1,2]. Among dietary components, phenolic compounds (PC) have been stand out as highly reactive and promising compounds since several mechanisms have been proposed to alleviate the symptoms or controlling the increasing prevalence[3]. However, the molecular interaction between dietary components (immunoreactive proteins/peptides and bioactives) and probiotic bacteria and the effect on bioaccesibility, bioavailavility and further bioactivities remain largely unexplored.

This study aims at characterizing the molecular interactions between PC, CD-related peptides and probiotic bacteria and further unravelling the impact on immunologic peptides bioaccesibility and overall antioxidant activity. Briefly, the molecular interactions at bacteria surface have been characterized by AFM and MALDI-TOF/TOF. Likewise, the effect of the green tea PC, epigallocatechin-gallate (EGCG) on biotransformation of the immunogenic peptide QLQPF(PQPQLPY)$_3$PQPQPF (32mer) by probiotic bacteria was followed by nano-LC-MS/MS.

AFM results highlighted the formation of large aggregates at cell surface resulting from the ability to EGCG to bind to bacteria mainly in the presence of immunogenic peptide. The aggregates identification by MALDI-TOF/TOF confirm the structural involvement of EGCG, 32mer and their metabolites, which clearly affected the bioaccessibility and biotransformation of the immunogenic peptide by probiotic bacteria. Furthermore, changes in antioxidant activity have been revealed. Since further studies are needed to verify the consequences in the immune response, these results open a new way to clearly understand the dietary components and probiotics interactions in a structural perspective as the basis for further recommend the use of probiotics in a nutrition precision approach. Indeed, additional studies claimed for evaluating the impact on bioactive compounds bioavailability, metabolism within different food sources considering the inter-personal variability. Besides, complex *in vitro* systems must be designed for a better understanding of mechanistic studies and the cellular impact of PC depending on anatomical locations (gut vs. blood) or intervention window (prevention vs. treatment).

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O 029 UNRAVELLING THE ANTI-ANGIOGENIC EFFECT OF DIETARY ISOFLAVONES AND THEIR CIRCULATING METABOLITES IN HUMAN AORTIC ENDOTHELIAL CELLS

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Background:

Angiogenesis refers to the formation of blood vessels from pre-existing vasculature and plays a key role in tumour growth and metastasis promoting proliferation and endothelial migration. Within the strategy of seeking novel anti-angiogenic drug candidates from natural sources, using phytochemicals such as dietary (poly)phenols is gaining research interest. Thus, isoflavones like genistein and daidzein show chemopreventive and anti-angiogenic effects in preclinical studies. However, these dietary molecules reach low concentrations in the bloodstream as they undergo gut microbial and phase-II metabolism, forming sulphates and glucuronides (i.e., equol 7-glucuronide) as the major circulating metabolites. Based on this metabolism, the microbial and(or) their phase-II metabolites might be the compounds that can modulate angiogenesis. However, whether the circulating metabolites and(or) their free forms are the active principles as anti-angiogenic molecules of the isoflavones-rich foodstuff remains elusive.

Objectives:

The main objective is to provide new insights into the anti-angiogenic role of dietary isoflavones and their derived metabolites. Specifically, considering the bioavailability and gut microbiota metabolism, we aimed to assess the effects of isoflavones and their derived metabolites on (i) tubulogenesis, (ii) endothelial migration, and (iii) the underlying molecular mechanisms. We also tested the capacity of human aortic endothelial cells (HAECs) to conjugate/deconjugate the molecules studied.

Methods:

HAECs were treated with genistein, daidzein, equol, or their phase-II metabolites (glucuronides and sulphates) using concentrations from 0.1 to 10 mM (similar to those detected *in vivo*), which lacked cytotoxicity. We first tested how the metabolites modulated the formation of capillary-like structures (tubulogenesis assay) in a time- and dose-dependent manner. We also investigated the effect of the molecules on endothelial migration (wound healing assay). The culture medium of these assays was analysed by UPLC-qTOF-MS to determine how HAECs metabolize the compounds tested. Finally, to assess the cellular targets of inhibition, we tested the effect of the isoflavones on the VEGF pathway (western blot and immunocytochemistry).

Results:

Genistein, daidzein, and equol exerted a dose-dependent inhibition on the capillary-like structure formation. The phase-II metabolism limited this capacity as only equol 7-glucuronide (at 10 mM) exerted a similar inhibitory effect. Equol and its glucuronide, but not genistein and daidzein, also exerted a dose-dependent reduction on HAECs´ migration. The effects on tubulogenesis and migration correlated with the ability of these compounds to hamper VEGF pathway activation. The deconjugation of the phase-II conjugates to free forms under our specific assay conditions could be relevant regarding the effects described.

Conclusion:

Our results provide significant insights into the efficacy of circulating isoflavone-derived metabolites targeting angiogenesis. These preclinical results require validation through further *in vivo* experiments to deepen our understanding of the role of isoflavones in the prevention/treatment of angiogenesis-related chronic diseases such as cancer and(or) atherosclerosis.

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O 030 CHARACTERIZATION OF THE INTER-INDIVIDUAL VARIABILITY ASSOCIATED WITH THE MICROBIAL METABOLISM OF (−)-EPICATECHIN

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Background:

Flavan-3-ols are the most consumed (poly)phenols in the Western diet. These compounds are known to prevent noncommunicable diseases such as obesity and cardiovascular diseases. It is now generally agreed that these beneficial effects are mediated by their interaction with the gut microbiota. In fact, flavan-3-ols are poorly absorbed in the small intestine and reach the colon where they are metabolized by specific bacteria into smaller bioavailable and potentially bioactive metabolites. However, there is a large inter-individual variability associated with the metabolism of flavan-3-ols by the gut microbiota. The latter could explain the heterogeneity of the results obtained from large clinical trials attempting to demonstrate the positive effects of these molecules on health.

To characterize this variability, the concept of metabotypes has been introduced to stratify individuals into groups with similar metabolic capacity. Previous studies successfully defined metabotypes associated with the metabolism of ellagitanins using a qualitative criterion (absence *vs* presence of specific metabolites). However, flavan-3-ols metabotypes are yet to be identified and cannot be defined using simple qualitative criteria, since all individuals can excrete a small amount of flavan-3-ols specific metabolites. Hence, studies have attempted to define these metabotypes using a quali-quantitative criterion (low *vs* high excretion) in urine samples. Recently, *in vitro* fecal fermentation was used to further understand the dynamics associated with metabolism of flavan-3-ols and assess their conversion rate.

Objectives:

The main objective of this study was to unravel the metabotypes associated with the metabolism of flavan-3-ols by the gut microbiota using *in vitro* fecal batch fermentation. More specifically, we aimed to define the metabotypes based on the metabolization rate of (−)-epicatechin and the resulting quali-quantitative metabolic profiles and to associate specific bacteria with each metabotypes.

Methods:

A total of 34 healthy subjects inoculated *in vitro* fecal batch fermentation supplemented with 100 µM of (−)-epicatechin and samples were collected every hour until 8 h and after 24 h. Targeted metabolomics was applied to quantify (−)-epicatechin and its microbial metabolites. Fecal microbiota was analyzed through 16S rRNA gene sequencing and short chain fatty acids were quantified to characterize the metabotypes in terms of fecal microbiota composition and function.

Results:

First, the subjects were separated into two metabotypes based on their metabolization rate of (−)-epicatechin. Fast converters completely metabolized (−)-epicatechin after 3 h of fermentation, while slow converters needed more than 7 h. Fast converters were associated with higher abundance of SCFA-producing bacteria, such as *Faecalibacterium spp.* and *Bacteroides spp.*, and their feces contained more SCFA. Secondly, the cohort was stratified into 3 metabotypes based on their capacity to produce higher quantity of specific metabolites. All subjects were able to convert (−)-epicatechin into 1-(3′,4′-dihydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)-propan-2-ol, but only half of them were able to significantly continue the degradation into 1-(3′-hydroxyphenyl)-3-(2″,4″,6″-trihydroxyphenyl)-propan-2-ol or 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone. New bacteria were linked to the production of these metabolites, notably *Adlercreutzia equolifaciens*, which was previously known to open (−)-epicatechin C-ring.

Conclusion:

We proposed robust flavan-3-ols metabotypes definitions which could be used to characterize intra-individual variability in clinical trials assessing the health effects of these molecules.

O 031 CITRUS EXTRACT HIGH IN FLAVONOIDS BENEFICIALLY ALTERS GUT MICROBIOTA METABOLIC RESPONSES IN HEALTHY SUBJECTS WITH FEATURES OF METABOLIC SYNDROME

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Background:

Flavonoids are polyphenolic compounds with various beneficial effects that include anti-inflammatory and anti-oxidative activities. Flavonoids present in citrus fruits, mainly hesperidin and naringin, are also able to interact with the intestinal microbiota and may thereby beneficially affect factors related to metabolic and intestinal health. For example, shifts in faecal microbiota composition and short-chain fatty acid (SCFA) profiles have been shown after treatment with citrus flavonoids. However, data about their effects on gut microbiota responses in metabolic health of human subjects, and in particular those with metabolic disturbances, is scarce.

Objectives:

The objective of this study was to investigate the effect of supplementation with a citrus extract rich in citrus flavonoids on gut microbiota metabolic responses in subjects with features of metabolic syndrome.

Methods:

Fifty healthy volunteers with two characteristics of metabolic syndrome were randomly allocated to groups receiving 500 mg citrus extract (MicrobiomeX®) or placebo for 12 weeks. Faecal SCFA and faecal calprotectin concentrations were measured at baseline and after 12 weeks of supplementation. In addition, fresh faecal samples of healthy volunteers with features of metabolic syndrome (n=7) were used to inoculate a validated *in vitro* model of the colon (TIM-2). Citrus extract was added to standard ileal efflux medium (SIEM) and was provided for three days at a concentration of 500 mg/day. Two untreated units with SIEM served as a control. SCFA production was analysed in lumen and dialysate samples obtained at 0, 24, 48 and 72 h.

Results:

In human volunteers, 12 weeks of daily supplementation with citrus extract resulted in a significant shift in the SCFA profile towards more production of butyrate (p=0.022) compared to the placebo group. Furthermore, there was a trend towards a reduction in faecal calprotectin levels, a marker for intestinal inflammation, compared to placebo (p=0.058). *In vitro*, continuous citrus extract supplementation also beneficially altered SCFA profiles. After citrus extract treatment, cumulative SCFA levels were increased compared to the SIEM control condition, which was mainly due to an increased production of butyrate, acetate and valerate.

Conclusion:

These results suggest that citrus extract intake may have a positive effect on gut metabolic responses and host health in healthy subjects with features of metabolic syndrome.

O 032 PREVIOUS VAGINAL DELIVERY OR BREASTFEEDING AND DIETARY STARCH AND AMINO ACIDS HAVE THE STRONGEST ENVIRONMENTAL ASSOCIATIONS WITH THE VAGINAL MICROBIOME IN PREGNANCY; SECONDARY ANALYSIS OF THE MICROBEMOM RANDOMISED CONTROLLED TRIAL

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Introduction:

The vaginal microbiome is a key player in reproductive health and is also intrinsically linked to gut microbial environment. There is strong evidence linking environmental exposures to gut microbial health, which in turn in increasingly evidenced to have a regulatory effect on phenotypic response in the vaginal microbiome. High quality environmental data describing associations between demographics, obstetric history, lifestyle and dietary pattern and vaginal microbiota is currently lacking. This study aimed to identify significant environmental factors that influence vaginal microbial health.

Methods:

This is a secondary analysis of the MicrobeMOM randomised controlled trial (Sept 2016- July2019). All women who had vaginal samples collected at randomisation (16weeks-gestation) were included. Shotgun sequencing of vaginal samples was performed and samples heat-mapped to assess species dominance. Shannon Index Alpha diversity was calculated. Baseline demographic and obstetric information, dietary data, wellness and exercise patterns were recorded. Ordination of vaginal species was performed and dietary and environmental covariates were examined using envfit analysis to show effect size and significance for each covariate, comparing difference in centroids of covariate relative to total variation. Significant covariates were then examined against vaginal alpha diversity and assignment to a vaginal community state type (CST). Statistical analysis was performed employing R Statistical Software (version 4.2.2, 2022-10-31). Ethical approval was granted by the National Maternity Hospital.

Results:

Previous vaginal delivery and history of breastfeeding significantly impacted vaginal species (Adjusted R^2 0.070, p=0.001; Adjusted R^2 0.053, p=0.005, respectively), and were associated with lower assignment to CST I (p=0.003, p=0.005, respectively), but did not impact alpha diversity (Mann-Whitney U=1553.0 and 488.0, p=0.942 and p=0.874, respectively).

Dietary intake of carbohydrate, maltose and dietary glycaemic load had significant impact on vaginal microbial species (Adjusted-R^2 0.057, p=0.038, Adjusted-R^2 0.061, p=0.033, Adjusted-R^2 0.065, p=0.022, respectively). Carbohydrates, starch and maltose correlated with increased alpha diversity (+0.001 per carb g, p=0.032; +0.002 per starch g; +0.044 per maltose g, p=0.043). CST IV was shown to correspond to significantly higher intakes of carbohydrates and starch and higher dietary glycaemic load compared to CST I and III. Dietary intake of amino acids lysine, leucine, valine and alanine (precursors to short-chain-fatty acids) had a significant impact on vaginal species (Adjusted-R^2 0.077, p=0.023, Adjusted-R^2 0.051, p=0.048, Adjusted-R^2 0.057, p=0.028, Adjusted-R^2 0.064, p value=0.018), but did not correlate with alpha diversity or assignment of CST.

No link was observed between previous caesarean section, non-starch based macronutrients (fibre and non-starch polysaccharides) or maternal exercise or wellbeing with vaginal species, diversity or CST assignment.

Conclusion:

This secondary analysis of the MicrobMOM randomised controlled trial reveals that previous vaginal birth, history of breastfeeding, glycaemic load and dietary intake of starch and Short-Chain-Fatty-Acid-Precursor amino acids have the most significant environmental impact upon vaginal microbial composition. These data provide an intriguing and novel snapshot into the impact of environment and diet on the vaginal microbiome and highlights the need for further investigation into the complex interactions between diet, human gut and vaginal microbiome.

O 033 REFINED DIETS ALTER BILE ACID PROFILES IN THE GUT AND THE BRAIN AND ARE CONCURRENT WITH INCREASES IN NEUROINFLAMMATORY SIGNALLING

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Over recent decades, modern dietary patterns have changed significantly due to the increasing availability of convenient, ultra-processed refined foods. Refined foods commonly have key nutrients and fibres removed, which has been associated with gut microbial dysfunction and the development of several deleterious health conditions. As the gut microbiome can communicate with the brain through a "microbiota-gut-brain axis", the consumption of refined foods has the potential to affect cognitive health. In this study, multi-omics approaches were employed to assess the effect of a refined diet on the microbiota-gut-brain axis, with a particular focus on bile acid metabolism. Mice maintained on a refined low-fat diet (rLFD) for eight weeks displayed significant gut microbial dysbiosis, as indicated by diminished alpha diversity and altered beta diversity ($p<0.05$), when compared to mice receiving a standard diet. These included significant modulation of gut microbiota that regulates bile acid metabolism, as indicated by changes in bacterial entities with bile salt hydroxylase and 7α-dehydroxylation capability. Changes in gut microbiota composition paralleled modulation of the metabolome, including a significant reduction in short-chain fatty acids (acetate, propionate and n-butyrate; $p<0.001$) and alterations in bile acid concentrations. Interestingly, the rLFD led to dysregulated bile acid ratios across both the colon (including increases in T-β-MCA: CA and UDCA: CA and decreases in TCA: CA; $p<0.05$) and the brain (including increases in α-MCA: CA and decreases in LCA: CA; $p<0.05$) which coincided with altered pro-apoptotic and neuroinflammatory gene expression. In particular, the concentration of taurine-conjugated bile acids (TCA, TDCA and T-α-MCA) was inversely correlated with the expression of *NF-κB1*, a key transcription factor in neuroinflammation. Overall, our results suggest a novel link between a refined low-fat diet and detrimental neuronal processes, likely in part through modulation of the microbiota-gut-brain axis and bile acid dysmetabolism.

O 034 ANTI-INFLAMMATORY POTENTIAL OF A SUPERCRITICAL FLUID EXTRACT FROM CHICORY ON THE INTESTINAL MUCOSA: IMPLICATIONS OF GUT MICROBIOTA METABOLIZATION

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Chicory (*Cichorium intybus L.*) is an under-valorized crop that is commercially exploited for isolation of the dietary fiber inulin. Inulin processing comprises removal and disposal of sesquiterpene lactones (SLs), a class of compounds with several reported bioactivities, including anti-inflammatory potential [1]. The main SLs found in chicory are lactucin, lactucopicrin, and their respective derivatives [2].

In this study, we showed that a supercritical fluid extract (SFE) obtained from chicory, which is rich in lactucin, 11β,13-dihydrolactucin, lactucopicrin, and 11β,13-dihydrolactucopicrin, exhibited anti-inflammatory activity in a physiologically relevant model of the inflamed intestinal mucosa, composed of enterocytes, goblet cells, and antigen-uptake facilitator microfold (M)-cells. In this model, 50 µg/mL SFE was able to exert an anti-inflammatory effect at different stages of cellular inflammatory response. In particular, SFE decreased gene and protein expression levels of iNOS and COX-2, assessed by RT-PCR and Western blot experiments, respectively. Two mucins (MUC2 and MUC5AC), which are important molecules contributing to the defense of the intestinal mucosa, were also assessed. However, treatment with SFE had no significant effect on the levels of MUC2 and MUC5AC, when compared to the inflamed control. Additionally, SFE led to a decreased gene expression of the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α. Consistently, ELISA assays demonstrated that the release of IL-8, which acts as a chemoattractant upon inflammation, was decreased following incubation with SFE. Such results might be explained by the ability of the extract to interfere with the MAPK and NF-κB pathways. In particular, SFE significantly prevented MAPK p38 phosphorylation, and decreased phosphorylation of the p65 NF-κB subunit to some extent, as detected by Western blot.

To better understand the stability of the SFE SLs after gut microbiota metabolism, SFE was submitted to an *in vitro* colon model [3], and SL composition was analyzed by LC-MS. After a 6-hour assay, microbiota metabolization led to a significant decrease of cysteine-SL conjugates, along with lactucopicrin and 11β,13-dihydrolactucopicrin. The levels of lactucin were observed to decrease, while 11β,13-dihydrolactucin levels increased, which suggests a conversion of the former into the latter. Samples obtained from the microbiota metabolization of SFE are being tested in the inflamed intestinal mucosa cell model, to assess the impact of metabolization on the anti-inflammatory effect of SFE.

This study is an important step forward in elucidating the anti-inflammatory mechanisms of uncharacterized chicory SLs for potential application in the treatment of IBD.

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Acknowledgements:

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O 035 IDENTIFYING NOVEL BIOLOGICAL ACTIONS OF THE MANGOSTEEN (*Garcinia mangostana*) IN PROSTATE AND COLON CANCER

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More than 80 xanthones have been identified from the mangosteen (*Garcinia mangostana).* Our lab has evaluated the biological activity of these xanthones in a variety of targets for prostate, breast and colon cancer through a combination of cell free, cell based and *in vivo* models. In prostate cancer cells we identified α-mangostin and gartanin, two of the more abundant xanthones in the mangosteen, as novel disruptors of androgen receptor activity. Interestingly, both xanthones induce apoptosis and disrupt androgen receptor functionality through distinct mechanisms ultimately leading to androgen receptor degradation. Gartanin was observed to interact directly with the ligand binding domain of the androgen receptor ultimately leading to androgen receptor degradation. Further analysis using surface plasmon resonance we determined that α-mangostin binds BiP, a chaperone protein, and contributes to androgen receptor degradation. Using a cell-based approach α-mangostin was found to promote androgen receptor unbiquitination via the proteasome using Western blots and immunoprecipitation. Next, we modified the androgen receptor using site directed mutagenesis to determine if mechanisms of resistance limited the activity of α-mangostin. Pharmacokinetic analysis was also performed with pure α-mangostin and a highly characterized mangosteen extract. *In vivo* data has confirmed that α-mangostin is effective at reducing tumor size in mice xenografted with prostate cancer cells more effectively than bicalutamide. In a second set of experiments using colon cancer cells we evaluated the effect of xanthones on key anti-inflammatory and anti-oxidative activities. In this set of experiments we isolated and identified inducers of aryl hydrocarbon receptor (AhR) and nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathways from mangosteen using a bioassay-guided strategy. Isolation of xanthones from active fractions identified seven isoprenylated xanthones was performed. H1L6.1c3 (AhR induction) and HepG2-ARE (Nrf2 induction) cells were utilized to establish structure activity relationships of xanthones for each target. Next, we evaluated epithelial barrier function and determined that garcinone D inhibited oxidative stress. These results suggest a dual function of garcinone D that promoted protection against epithelial barrier dysfunction. In summary, these data highlight how the orientation of key functional groups on xanthones contribute to novel biological mechanisms in prostate and colon cancer cells.

O 036 BLACK SOLDIER FLY LARVAE AS A NEW SUSTAINABLE SOURCE OF DIETARY BIOACTIVES

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The incorporation of edible insects in animal and human food is one of the solutions proposed to deal with the depletion of resources and to help fight against global warming. The consumption of insects by certain farm animals, e.g. chickens and fish, is not new, nor is entomophagy. Indeed, more than 2 billion inhabitants consume insects regularly in Asia, Africa and south America. Raising edible insects has many advantages, e.g. reduced water requirements and greenhouse gas emissions. Of the approximately 2000 listed species of edible insects, only a handful are authorized for use in animal and human nutrition, e.g. black soldier flies and mealworms. These species are raised in particular with the aim of producing proteins of high nutritional quality which are considered as a more sustainable alternative to soy and fish proteins. But insects do not only contain proteins, they also contain lipids, micronutrients and phytochemicals. One of the interests of insects is that their body composition is much more affected by their diet than that of livestock. This can be a drawback, such as the bioaccumulation of chemical contaminants that may be present in their diet, but it can also be a benefit. Indeed, this makes it possible to envisage making them bioaccumulate compounds of nutritional interest. It is on this basis that we have developed a project in the laboratory aimed at evaluating the ability of black soldier fly larvae (BSFL, *Hermetia Illucens*) to bioaccumulate fat-soluble vitamins and carotenoids. We used this insect because it has the enormous advantage of being able to grow on a wide variety of organic substrates, and this is what we needed because the substrates rich in different micronutrients are very varied. We therefore did a series of studies in which we raised BSFL on substrates rich in provitamin A (β-carotene, α-carotene, β-cryptoxanthin), e.g. carrots, sweet potatoes, pumpkins, or in lutein, e.g. kale, broccoli, parsley, or in vitamin E (α and ɣ-tocopherol), e.g. rice bran, corn cake. We then observed that the larvae could bioaccumulate concentrations of these compounds often equal to, and sometimes higher than, those of the substrates on which they had been reared. We were also able to show, thanks to an *in vitro* digestion model coupled with caco-2 cells, that the bioavailability of these lipophilic compounds in the larvae was of the same order of magnitude as that observed in plants, sometimes it was even higher than in the corresponding substrate. Finally, a study in an animal model, the gerbil, has shown that larvae enriched in provitamin A have the ability to restore a vitamin A deficient status in these animals. Our results show that it is possible to obtain BSFL rich in fat-soluble vitamins and carotenoids. This opens an exciting field of research to find out if other insect larvae, e.g. mealworms (*Tenebrio Molitor*), have the capacity to bioaccumulate these compounds and if edible insects can bioaccumulate other food bioactives, e.g. polyphenols, glucosinolates, phytoestrogens, and whether these bioactives are bioavailable in the insect matrix.

O 037 MYCOTOXINS IN OUR DIET: DEEPENING THE KNOWLEDGE MINIMIZES THE HEALTH RISK (?)

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Various toxic compounds lurk in food and feed, posing a threat to both humans and animals. Among the most widespread natural toxins belong mycotoxins, the secondary metabolites produced by toxinogenic microscopic filamentous fungi of the genus *Aspergillus, Penicillium, Fusarium, Alternaria*, and others. Along with the changing climate, shift in our food system towards a more plant-based diet, and the growing popularity of various plant-based dietary supplements, the contaminated crops pose a significant health risk to humans. Within the lecture, two mycotoxin-associated food safety challenges, particularly (i) co-occurrence of multiple mycotoxins in dietary supplements intended for treatment of liver, and (ii) problematics of "masked" mycotoxins as conjugates of (poly/oligo)saccharides in biotechnology products, will be introduced. As one of the main reasons associated with high levels of glycosylated mycotoxins in sprouted cereals and malt is the development of fungal infection on a waterized substrate, there is an urgent need to find suitable decontamination ways. In this respect, the latest research performed at UCT brings interesting pilot findings about the effect of 'pulsed electric field' technology (PEF) on minimization of viability of *Fusarium* pathogens on cereals, as well as destruction of free/glycosilated mycotoxins. During the presentation, possibilities of metabolomics (stand alone or with other "omics" disciplines) for demonstration of fungal pathogen(s) manifestation will be showed, and importance of sensitive analytical methods based on high-resolution mass spectrometry will be demonstrated.

O 038 PYRROLIZIDINE ALKALOIDS IN *Borago officinalis*: PROFILING AND QUANTIFICATION

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Borago officinalis L. (borage) is consumed in herb blends, salads, and food supplements. As many plants from the Boraginaceae family, it produces pyrrolizidine alkaloids (PA). PA are plant toxins posing a safety risk to humans due to their liver toxicity and genotoxic potential. In 2020, the European Union (EU) set maximum levels for 35 PA in specific foodstuffs. The maximum level for the sum of PA in fresh and frozen borage leaves is 750 µg/kg [1].

We conducted a detailed profiling of PA in borage leaves and flowers by UHPLC-QToF analysis. Subsequently, a LC-MS/MS method for individual quantification of PA found in borage was developed. Method development also included an easy and fast extraction procedure, which was optimized using a *design of experiment* (DOE) approach. The most efficient extraction was achieved using 0.2% formic acid in 10% methanol at a temperature of 47.5 °C for 60 min. The final method was validated according to SANTE guideline, showing good accuracy (recoveries 85–121%) and precision (RDS≤11%) [2].

PA contents and profiles of borage leaves and flowers were determined in different sample sets. First, borage was grown in a climate chamber (n=6) [2]. Secondly, young plants were given to private garden owners for further cultivation under a wide range of conditions. Plants were harvested after 5–7 weeks (n=28) or 12–14 weeks (n=11) of garden cultivation, respectively. When present, flowers were analyzed in addition to the leaves (n=23). Thirdly, we received borage samples from horticulture companies, who professionally produce borage plants for sale (n=14).

Levels of toxicologically relevant 1,2-unsaturated PA in borage leaves ranged widely. For plants grown in the climate chamber, small (<14 cm) and large (>14 cm) leaves were analyzed separately. PA contents of the large leaves were 1,678–4,967 µg/kg fresh matter (median: 2,503 µg/kg), while small leaves had approx. 20 times higher PA contents of 37,067–118,303 µg/kg fresh matter (median: 54,962 µg/kg). PA contents in older plants from cultivation in private gardens were substantially lower and ranged between 01,241 µg/kg fresh matter (median: 49 µg/kg). Approx. 18% of garden plants did not contain PA above the *limit of quantification* (LOQ). In all but one flowering plants, the PA content of flowers was higher than the corresponding PA content of the leaves. PA contents in the leaves of non-flowering, professionally grown plants ranged widely (39935,330 µg/kg fresh matter; median: 6,077 µg/kg).

Analyses showed that acetyl-lycopsamine-*N*-oxide, lycopsamine-*N*-oxide, and amabiline-*N*-oxide were the quantitatively most relevant PA in the majority of borage leave samples, accounting for 69–100% of the total content. However, of these analytes only lycopsamine-*N*-oxide is listed in the EU Regulation and, therefore, is considered in the assessment of maximum levels. We showed that on average approx. 70% of PA present in borage leaves are not covered by the EU Regulation.

[1] Commission Regulation (EU) 2020/2040 of 11 December 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of pyrrolizidine alkaloids in certain foodstuffs.

[2] M. Sattler, V. Müller, D. Bunzel, S.E. Kulling und S.T. Soukup, Talanta 2023, 258, 124425. DOI: 10.1016/j.talanta.2023.124425.

O 039 SHORT-TERM *Aronia melanocarpa* EXTRACT SUPPLEMENTATION IMPROVES COGNITIVE PERFORMANCE: A RANDOMIZED CONTROLLED TRIAL IN HEALTHY YOUNG ADULTS

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Background and objectives:

Supplementation with various anthocyanin-rich foods has already been shown to exert beneficial effects on cognitive performance. Furthermore, we have recently observed beneficial effects of long-term Aronia Melanocarpa- an anthocyanin-rich berry- extract (AME) supplementation (16 mg anthocyanins/day) on psychomotor speed in healthy middle-aged adults. However, potential effects of a higher dose of AME in a short-term setting, as well as in a younger population remain unknown. The objective of this study was therefore to investigate the short-term effect of AME supplementation on cognitive performance in healthy young adults. Also, effects on potential underlying mechanisms, such as improved peripheral vascular function and circulating brain-derived neurotrophic factor (BDNF) concentrations were addressed.

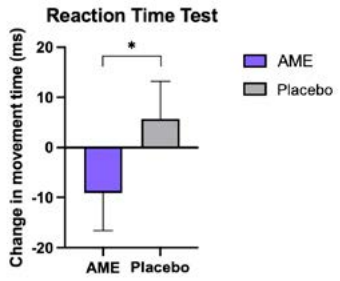
Methods:

A randomized, double-blind, placebo-controlled, cross-over study was performed involving a total of 35 apparently healthy 18–35 year old adults. Participants consumed AME or a placebo for one week, separated by a wash-out period of two weeks. The AME extract corresponded to an anthocyanin intake of 180 mg/day. Cognitive performance was measured using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Arterial stiffness (pulse wave velocity), blood pressure, retinal microvascular calibers (fundus photography) and BDNF concentrations were also assessed before and after the intervention and placebo period.

Results:

Study participants were 25 ± 4 years old with an average BMI of 23.4 ± 2.7 kg/m^2. A significantly reduced movement time of 4.8% within the five-choice reaction time test was observed after one week of AME supplementation, compared to placebo (treatment effect of -11.56 ms; $p<0.05$). Memory and executive function tests did not change. Moreover, serum BDNF concentrations were significantly higher after AME supplementation, compared to placebo (+5.7%; treatment effect of 1.8 ng/mL; $p<0.05$). However, peripheral vascular function markers were not affected.

Figure 1: The change in movement time during the reaction time test after one week of AME supplementation[1].



[1] Data are presented as mean change from baseline ± standard error of the mean. Analysis was performed with a linear mixed model using intervention, period, and gender as fixed factors. * indicates $p<0.05$.

Discussion:

One week of AME supplementation improved cognitive performance reflected by a shorter movement time on the five-choice reaction time test. Increased BDNF concentrations might be related to this short-term effect observed in the domain of attention and psychomotor speed in healthy young adults. However, no changes were observed in peripheral vascular function.

O 040 PRODUCTION AND EVALUATION OF RARE SUGARS AND GLYCOSIDES AS NEW FOOD INGREDIENTS

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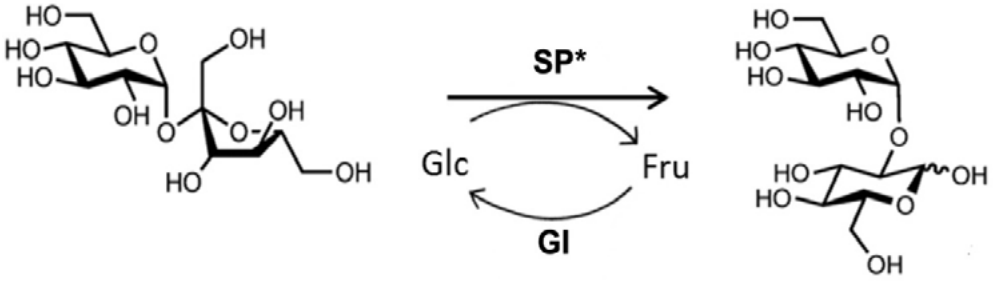
Although table sugar (i.e. sucrose) has gained somewhat of a bad reputation, plenty of healthier alternatives can be found in nature. In honey, for example, nine rare sugars have been identified, including the most potent prebiotic known to date (i.e. kojibiose). Since these compounds are only present in small amounts, their large-scale extraction from natural sources is not a viable option. Instead, dedicated production processes must be developed to enable their application in the food industry. By means of semi-rational mutagenesis, we were able to create selective enzymes for the conversion of sucrose into glucobioses with either an α-1,2-linkage (kojibiose) [1] or an α-1,3-linkage (nigerose)[2]. Furthermore, by varying the acceptor substrate (replacing Glc with Gal, Xyl, etc.) we could further diversify the portfolio of alternative sugars [3]. Kojibiose has already been produced at ton-scale (Fig. 1) and others are coming soon. In turn, by rationally expanding the size of the enzyme's active site, a whole range of novel antioxidant glycosides could be synthesized in gram amounts [4]. These products display improved solubility and stability, which should be beneficial for use in food or cosmetic formulations.

Figure 1: Transglucosylation of sucrose into kojibiose. SP* = engineered sucrose phosphorylase



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O 041 PROTOCOLS FOR INVESTIGATING OF THE PRODUCTION OF COLONIC CATABOLITES OF DIETARY (POLY)PHENOLS

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Following ingestion of fruits, vegetables and derived products, sizeable amounts of (poly)phenols can pass into the distal gastrointestinal tract. There they undergo microbiota-mediated ring fission resulting in the production of a diversity of low molecular weight phenolic catabolites some of which also appear in the circulatory system and are excreted in urine as phase II metabolites. These is increasing interest in these catabolites because of their bioactivity and use as biomarkers of (poly)phenol intake. Investigating the fate of dietary (poly)phenolics in the colon is complicated by a number of factors, among them is the fact some phenolic catabolites are also produced from surplus amino acids in the body in reactions catalysed by endogenous mammalian enzymes. Protocols will be discussed that can be used in bioavailability studies to distinguish these background phenolics from those arising from dietary (poly)phenols.

O 042 MOLECULAR EFFECTS OF SILYMARIN FLAVONOLIGNANS: CHIRALITY IS PIVOTAL IN BIOLOGICAL ACTIVITY

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Silymarin (extract from the milk thistle fruit - *Silybum marianum*) has traditionally been used in various medicinal applications since ancient times. Controversies often arise, mainly due to the non-standard composition of this phytopreparation, the use of various undefined mixtures, the misattribution of silymarin *vs*. silybin, and the failure to consider the chemistry of the individual components of silymarin [1]. A major component of silymarin is the flavonolignan silybin [2] and its congeners isosilybin, silychristin, silydianin, and the dehydroderivative 2,3-dehydrosilybin. All these compounds (except silydianin) occur in nature as two diastereomers [3]. Since their isolation in 1959, silybin and other flavonolignans have attracted increasing interest, leading to the publication of ca 500 research articles per year (in the last 10 years).

We will show that the specific activities of the respective diastereomers of flavonolignans and also of the enantiomers of their 2,3-dehydro derivatives differ significantly in the 3D anisotropic systems (typically biological systems). *In vivo*, silymarin flavonolignans do not act as redox antioxidants - silybin is even a very poor antioxidant - but they play a role as specific ligands of biological targets following the "lock-and-key" concept. This also allows the separation of the respective diastereomers by enzymatic methods [4].

Estrogenic, antidiabetic, anticancer, antiviral, and antiparasitic effects will be demonstrated in optically pure flavonolignans. Potential applications of pure flavonolignans have also been shown in cardiovascular and neurological diseases. Inhibition of drug metabolizing enzymes and modulation of multidrug resistance by these compounds are discussed in detail [5,6]. The future of "silymarin applications" lies in the use of optically pure components that can be applied directly or used as valuable lead structures, and in the exploration of their true molecular effects.

Figure 1: Silybin A and silybin B

Acknowledgements:

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O 043 USE OF *Callistemon citrinus* EXTRACTS FOR THE PRODUCTION OF BIOACTIVE PACKAGING

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Background:

Nowadays flowers, besides their traditional uses, are becoming quite popular and gaining attention in the food sector worldwide, especially for their bioactive molecules. Further, some flower extracts or components have been used for the production of bioactive materials[1]. In this regard, our study is focused on the production of bioplastics functionalized using extracts from *Callistemon citrinus* (also known as Lemon bottlebrush) which is one of the most common ornamental plants worldwide[2] and characterized by the presence of intense red coloration of its petals.

Objectives:

The idea of this work is to convert these bioactive extracts into the formulation of bio-based packaging materials. Hence, hydroplastic chitosan-based materials were prepared with *Callistemon citrinus* extract. The films were prepared as a function of different concentrations of extracts and their antioxidant activity was evaluated[3]. In particular, the bioplastics will be applied to selected fruits and vegetables.

Methods:

The isolated bioactive molecules from *Callistemon citrinus* flowers were identified and quantified by RP-HPLC-DAD and estimating the antioxidant activity with the main antioxidant assays (DPPH). To produce the bioplastics the film forming solutions (FFSs) in the absence and presence of different percentages of the extracts were cast and dried onto Coatmaster. The obtained bio-composite were characterized according to mechanical properties, barrier features towards H_2O, CO_2,O_2, moisture content and moisture uptake. Investigations on film opacity and color were also considered.

Results:

The RP-HPLC-DAD analyses show the presence of only four anthocyanins bioactive molecules, named cyanidin-3,5-O-diglucoside (cyanin), peonidin-3,5-O-diglucoside (peonin), cyanidin-3-O-glucoside, cyanidin-coumaroylglucoside-pyruvic acid. Moreover they showed very interesting antioxidant activity in all the performed assays. Starting from these results, the obtained films showed an increase in moisture content and uptake by the addition of extract. We have proven that the extract functionalized materials are endowed with high antioxidant activity. The film mechanical properties showed that the extract enhances extensibility and at same time reduces the tensile strength. The films water vapor (WV) and gas (CO_2 and O_2) permeabilities were investigated and compared to the values obtained by analyzing a commercial material, low-density polyethylene (LDPE). The permeability to CO_2 was lowered by the flower extract likely due to the structural changes by the addition of polyphenols in the chitosan matrix. However, the water vapor permeability was not significantly altered. The opacity values of the films increase by increasing the extract amount. However, the color of the films showed that all the films possess a color in the range of light brown.

Conclusion:

The obtained results have demonstrated the *Callistemon citrinus* extract is able to make the film more flexible and less rigid. In the same manner, the bioactive compounds influenced the film moisture content and uptake. Above all, it is worthy of note that the functionalized films had a higher antioxidant activity making them a good candidate as edible packaging to preserve the shelf-life of different fruits and vegetables. Further investigations regarding their effect on real food will be also evaluated.

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O 044 IMPACT OF FOOD STRUCTURE ON THE BIOACCESSIBILITY OF BERRY (POLY)PHENOLS

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(Poly)phenol bioaccessibility is of importance to understand their beneficial effects on human health, as it determines the extent to which they are released after ingestion. Complex food matrices and cellular barriers such as plant cell walls are considered critical restrictive factors. However, little evidence exists on the impact of these structures on the bioaccessibility of blueberry (poly)phenols at different stages of digestion and the role of food processing strategies.

This study investigated the localisation, release, and metabolism of fresh and frozen-thawed blueberry (poly)phenols within fruit tissues during oral processing and gastrointestinal digestion, and how the resulting changes in matrix structure affect (poly)phenol bioaccessibility. An *in vivo* human mastication study was conducted with 17 healthy participants to evaluate the oral processing of berry (poly)phenols, while the INFOGEST protocol was used to simulate the gastric and duodenal digestion *in vitro*. In addition, the effect mechanical processing was assessed in the form of a blended blueberry smoothie using the *in vitro* digestion protocol. Particle size distribution of berry samples, which was obtained after human and simulated mastication and from blended smoothie was evaluated by wet sieving fractionation. Bioaccessible (poly)phenols were identified and quantified by UPLC-QqQ-MS/MS and their bioaccessibility was calculated. Structural characteristics of digested blueberry tissues as well as the localisation of (poly)phenols were determined by epifluorescence microscopy.

Overall, 21 anthocyanins, 35 flavonoids, 14 phenolic acids, and their derivatives were found in blueberry samples. Most blueberry (poly)phenols including anthocyanins were concentrated in the vacuole of skin cells. The rupture of cellular barriers not only generated the release of (poly)phenols, but also resulted in the movement of (poly)phenols towards the plant cell walls. Processing by mechanical shearing (including simulated mastication and blending) produced significantly smaller particle size and higher release of (poly)phenols than human mastication. The freeze-thaw process altered the matrix structure of blueberry tissue through the growth of intracellular ice crystals. (Poly)phenol bioaccessibility following gastric digestion (~ 20%- 50%) was significantly higher than that in the oral phase (~ 10%), and anthocyanins were much more stable in the gastric environment. However, after duodenal digestion, anthocyanin recoveries were significantly reduced to less than 10%, likely due to the high pH environment in the duodenal phase relative to the gastric environment, as well as the effect of bile salts and digestive enzymes.

In conclusion, bioaccessible (poly)phenols reached their highest concentration after gastric digestion and was significantly reduced after duodenal digestion. Food processing strategies such as mechanical shearing and freezing generate smaller blueberry particles and higher (poly)phenol bioaccessibility than fresh blueberries following human mastication. These results indicate that mechanical simulation of oral processing used in *in vitro* digestion protocols should be interpreted with caution.

O 045 ASSESSING THE IMPACT OF A (POLY)PHENOL-RICH DIET ON BIOMARKERS OF INTESTINAL PERMEABILITY AND INFLAMMATION IN OLDER ADULTS: THE MaPLE RANDOMIZED CONTROLLED TRIAL

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Ageing process often involves a gradual decline in the integrity and functionality of the intestine, which has been attributed to dysregulated immune system activation leading to systemic inflammation and impairment at the epithelium level. While preclinical research has shown the potential of phenolic compounds in regulating intestinal permeability and related inflammatory processes, human studies on this topic remain scarce. Within the MaPLE study, we tried to demonstrate that the intake of a (poly)phenol-rich diet (PR-diet) could affect important targets and pathways related to IP, such as serum zonulin and blood microbial DNA in older subjects. In the present study, we have tried to identify further biomarkers of IP and systemic inflammation and also to investigate their modulation following the PR-diet.

A randomized, controlled crossover study was conducted on 51 older individuals (≥60 years) with increased IP, as determined by serum zonulin levels. Participants were assigned to a control diet (C-diet) or a PR-diet for 8 weeks each, with an 8-week washout period in between. The levels of several biomarkers related to IP, inflammation, and endothelial dysfunction were further measured through ELISA kits using serum and fecal samples (ZO-1, occludin, adiponectin, calprotectin, fecal calprotectin, sCD14, IL-6R, and VE-cadherin levels). At baseline, the relationship among biomarkers was identified through Spearman correlation and network correlation analyses.

Based on network correlation analysis, two clusters of subjects were identified: one cluster with high levels of serum calprotectin, fecal calprotectin, soluble cluster of differentiation 14 (sCD14), interleukin (IL)-6, tumor necrosis factor (TNF)-α, C-reactive protein (CRP) and bacterial DNAemia (16S rRNA gene copies), with potential inflammatory-induced intestinal permeability; while the other cluster had high levels of serum occludin, IL-6 receptor (IL-6R), soluble intercellular adhesion molecule-1 (sICAM-1) and vascular endothelial (VE)-cadherin, with potential inflammatory-induced endothelial dysfunction. A significant treatment × time interaction was observed for serum calprotectin levels, which decreased after the PR-diet. Additionally, tight junction protein zonula occludens-1 (ZO-1) levels increased after both the PR-diet and C-diet.

Overall, this study provides further support to the hypothesis that a diet rich in (poly)phenols may help improve intestinal permeability-associated conditions. In this regard, calprotectin could play a pivotal role being a protein that typically increases with age and is considered indicative of immune system activation and systemic inflammation.

Keywords:

Ageing; Leaky gut; Inflammation; Flavonoids; Phenolics; Diet.

O 046 CHARACTERIZATION OF THE URINARY PROFILE OF PHENOLIC METABOLITES OF POSTMENOPAUSAL WOMEN AFTER DIET SUPPLEMENTATION WITH CHOCOLATE, GREEN TEA AND FRUIT JUICE

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Menopause is a critical stage in woman's life, in which cardiometabolic alterations appear, such as insulin resistance or a predisposition to visceral fat deposits, leading to an increased risk of cardiovascular diseases (R-CVD). Different pharmacological therapies such as hormone replacement have been designed to reduce the effects of menopause. However, they are associated with an increase in other pathologies such as some types of cancer. For this, new therapies and strategies to reduce the R-CVD in postmenopausal women using natural compounds with little or no adverse effects are desirable. In this sense, plant-based diets rich in fruit and vegetables could play a fundamental role in the reduction of R-CVD due to its high content in bioactive compounds such as (poly)phenols, which are recognized by its antioxidant, anti-inflammatory and vasodilator activity.

The main aim of this research was to characterize the phenolic metabolite profile generated after diet supplementation of postmenopausal women with (poly)phenol-rich foods, in order to correlate it with changes in the gut microbiota towards profiles having a potential beneficial effect on R-CVD. A clinical trial was developed (NCT05255367) with a control period and an experimental period, where the diet of postmenopausal women was supplemented for 2 months with 16.6 g of dark chocolate and a cup of green tea due to their high flavan-3-ol content, and a 100 ml of a fruit juice of berries, pomegranate and orange, due to their content in anthocyanins, ellagitannins and ellagic acid, and flavanones, respectively. The supplementation provided a total of 620 mg of (poly)phenols per day, mainly represented by 68% flavan-3-ols, 20% hydrolysable tannins, 5% flavanones, 3% anthocyanins, 3% flavonols and 1% phenolic acids. At the beginning and at the end of each period, urine samples over 24 h were collected, and (poly)phenol metabolites were identified and quantified by UHPLC-MS/MS.

A total of 116 phase II conjugated metabolites were identified and quantified in urine after the supplementation period, mainly represented by 38% phenyl-γ-valerolactones, 45% phenolic acids (cinnamic acids, phenylpropanoic acids, phenylacetic acids, benzoic acids, and benzenetriols), 10% flavanones, 4% urolithins, 2% flavones and 1% flavan-3-ols. A significant increase in the excretion of total phenolic metabolites was observed from a mean value of 238 ± 155 µmol at the beginning of the intervention to 361 ± 161 µmol at the end of it. This increase in the phenolic excretion was mainly due to a significant increase in the excretion of phenyl-γ-valerolactones derived from the metabolism of the flavan-3-ols present in dark chocolate and green tea, an increase in the excretion of phenolic acids that may come from different (poly)phenol sources, and an increase in the excretion of urolithins derived from the metabolism of ellagitannins present in pomegranate.

The results obtained in this research will allow us to correlate the phenolic metabolite profile with the main changes in gut microbiota profile and better understand the impact of (poly)phenols on R-CVD of postmenopausal women.

O 047 EVALUATION OF URINARY PHENYL-VALEROLACTONES AS BIOMARKERS OF DIETARY FLAVAN-3-OL EXPOSURE

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9 *Danish Cancer Society, The Danish Cancer Society Research Centre, Copenhagen, Denmark*

Background:

Phenyl-γ-valerolactones (PVLs) have been proposed as potential biomarkers of dietary flavan-3-ol exposure.

Objective:

To investigate the performance of a range of PVLs as biomarkers indicative of flavan-3-ol intake.

Methods:

We report results of two companion studies. In the first study, 16 healthy participants consumed flavan-3-ol rich interventions (of either apple, cocoa, black tea, green tea, or a water [control] for 1 day each) in a randomised crossover design. Both 24-hour urines and first morning voids were collected with diet standardised throughout. For each participant, one intervention period was extended (to 2 days) to monitor PVL kinetics following repeat exposure. In the second study, 86 participants consuming their regular diet collected 24-hour urines and weighed food diaries from which flavan-3-ol intake was estimated using Phenol-Explorer. Ten PVLs were quantified using liquid chromatography tandem mass spectrometry.

Results:

In both studies, 5-(3′-hydroxyphenyl)-γ-valerolactone-4′-sulfate and putatively identified 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-glucuronide were the principal compounds excreted (**Figure 1, Panels B & D**). In the RCT, the sum of these PVLs was significantly higher than the water (control) following each intervention; individually, there was a shift from sulfation towards glucuronidation as the total excretion of PVLs increased across the different interventions (**Figure 1, Panel A**). In the extended RCT intervention period, no accumulation of PVLs was observed after consecutive days of treatment. Results were consistent, whether compounds were measured in 24-hour or first morning urine. In the observational study, the sum of the principal PVLs correlated dose-dependently (R_s=0.37, P=0.0006) with dietary flavan-3-ol intake, with similar associations for each individually, demonstrating their applicability to epidemiological settings (**Figure 1, Panel C**).

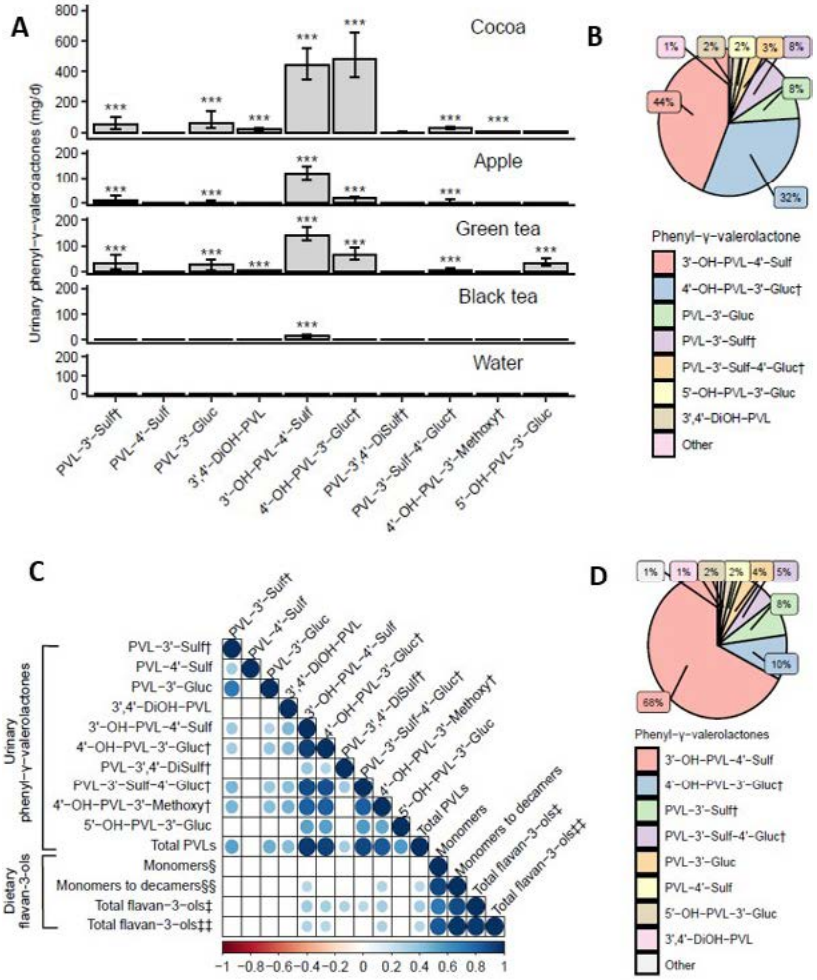

Figure 1: Panel A) Median [IQR] excretion of phenyl-γ-valerolactones in 24-hour urine following flavan-3-ol rich interventions of cocoa, apple, green tea, black tea or a water control in a crossover trial (n=16). Differences in phenyl-γ-valerolactone excretion across interventions assessed by linear mixed model on log-transformed data, when applicable. Stars highlight significantly higher excretion of specific phenyl-γ-valerolactones, after intake of the different interventions, in comparison to the water control. *P≤0.01, **P≤0.001, ***P≤0.0001. **Panel B)** Shows the average percent excretion of the measured phenyl-γ-valerolactones across interventions. **Panel C)** Correlation heatmap between dietary flavan-3-ol intake and 24-hour urinary phenyl-γ-valerolactone excretion in a cross-sectional study (n=86). Blue circles show the strength of significant positive correlations; blank (white panels) are non-significant correlations. Correlations calculated using Spearman's coefficient. P<0.01 was considered significant. **Panel D)** Shows the average percent excretion of the measured phenyl-γ-valerolactones in the observational study. Abbreviations: 5-phenyl-γ-valerolactone-3′-sulfate, PVL-3′-Sulf; 5-phenyl-γ-valerolactone-4′-sulfate, PVL-4′-Sulf; 5-phenyl-γ-valerolactone-3′-glucuronide, PVL-3′-Gluc; 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, 3′,4′-DiOH-PVL; 5-(3′-hydroxyphenyl)-γ-valerolactone-4′-sulfate, 3′-OH-PVL-4′-Sulf; 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-glucuronide, 4′-OH-PVL-3′-Gluc; 5-phenyl-γ-valerolactone-3′,4′-disulfate, PVL-3′,4′-DiSulf; 5-phenyl-γ-valerolactone-3′-sulfate-4′-glucuronide, PVL-3′-Sulf-4′-Gluc; 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-methoxy, 4′-OH-PVL-3′-Methoxy; 5-(5′-hydroxyphenyl)-γ-valerolactone-3′-glucuronide, 5′-OH-PVL-3′-Gluc.

§Monomers: sum of (epi)catehins, (epi)gallocatechins and their galloyl substituted derivatives.

§§Monomers to decamers: sum of the above plus 02 mers to 10 mers [excludes >10 mers].

‡Flavan-3-ol intake: sum of (epi)catehins and their galloyl substituted derivatives plus 02 mers [excluding prodelphinidins] to >10 mers.

‡‡Additionally includes (epi)gallocatehins, their galloyl substituted derivatives, plus prodelphinidins.

†Putatively identified compound.

Conclusion:

Urinary 5-(3′-hydroxyphenyl)-γ-valerolactone-4′-sulfate and putatively identified 5-(4′-hydroxyphenyl)-γ-valerolactone-3′-glucuronide are recommended biomarkers for dietary flavan-3-ol exposure.

O 048 POLYPHENOL OXIDASE AND BIOAVAILABILITY OF FLAVAN-3-OLS FROM FRUIT SMOOTHIES: IMPORTANCE FOR FOOD PREPARATION AND DIETARY ADVICE

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Background:

Flavan-3-ols are bioactive compounds found in a variety of fruits and vegetables (F&V) that have been linked to positive health benefits. Increasing habitual flavan-3-ol intake is challenged by the generally low consumption of F&V. While smoothies are a commonly endorsed, consumer-accepted means to increase the daily intake of these important foods, fruits used for smoothie preparation can have a high polyphenol oxidase (PPO) activity and thus potentially affect the content and bioavailability of flavan-3-ols.

Objective:

To assess whether or not consuming freshly prepared smoothies made with different PPO-containing fruit impacts the bioavailability of the flavan-3-ols.

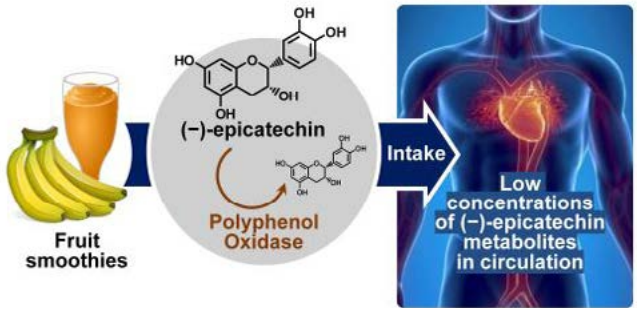

Figure: Impact of polyphenol oxidase on the bioavailability of flavan-3-ols in fruit smoothies.

Methods:

A controlled, single blinded and cross-over study was conducted in healthy men (n=8) who consumed a flavan-3-ol-containing banana-based smoothie (high PPO activity), a flavan-3-ol-containing mixed berry smoothie (low PPO activity) and flavan-3-ols in a capsule format (PPO-free control). A subsequent study consisted in a controlled, single blinded, cross-over, and randomized study in which healthy men (n=11) consumed a flavan-3-ol drink and a banana-based smoothie (high in PPO) that were prepared separately but consumed simultaneously by alternating sips from each beverage, and a flavan-3-ol drink without any banana PPO that served as a control. Flavan-3-ol metabolites were measured with validated UPLC-MS methods using authentic standards.

Results:

The peak plasma concentration (C_{max}) of flavan-3-ol metabolites after control intake was 680 ± 78 nmol/L, which was similar to the levels detected after the intake of the mixed berry smoothie (low PPO activity). In contrast, the intake of the banana smoothie (high PPO activity) resulted in a C_{max} of 96 ± 47 nmol/L, which was 89% lower than that after control. As expected, these results were partially explained by a rapid, PPO-dependent decay of flavan-3-ols in banana smoothie after preparation (half-life: 9.8 ± 2.1 min). To determine if banana PPO could also affect the bioavailability of flavan-3-ols after ingestion, a subsequent study was conducted in which flavan-3-ols were co-ingested with a high-PPO banana smoothie but preventing contact prior to intake. The results obtained showed that, compared to control, there was a reduction of 37 ± 6 % in flavan-3-ol circulating levels when flavan-3-ols were co-ingested with a high-PPO banana drink but preventing contact prior to intake. Consistent with these findings, banana PPO proved to remain active after incubation for 2 h under simulated gastric digestion conditions, thus suggesting that PPO can still react with flavan-3-ols in the stomach post-intake. Since PPO occurs widely in fruits and vegetables, PPO activity was measured in different fresh fruit and other plant-derived ingredients used for smoothie preparation. In addition to banana, pome fruits and leaves of beetroot were the foods with the highest PPO activity, and all had the ability to use (–)-epicatechin as an enzyme substrate.

Conclusions:

Bioavailability of flavan-3-ols, and most likely other dietary polyphenol bioactives, can be reduced substantially by the co-ingestion of high PPO-containing smoothies, the implications of which are of importance for dietary advice and food preparation both at home and in industrial settings.

O 049 EXAMINING WAYS TO IMPROVE SLEEP QUALITY AND SUPPORT HEALTHY AGEING IN OLDER ADULTS WITH SLEEP DISTURBANCES THROUGH TARGETING THE GUT MICROBIOME WITH SAFFRON SUPPLEMENTATION

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Age-related neurodegenerative diseases are a growing societal problem with numerous repercussions. Lifestyle and environmental factors play a key role in their development, with sleep quality being one of the major contributors to age-related cognitive decline and dementia. Insomnia symptoms tend to increase with age, with prevalence rates approaching 50% in adults aged 65 and over, hence strategies to improve sleep quality in older people are essential. The gut-brain axis plays an important role in health and disease, with recently reported impacts on healthy ageing. Both sleep quality and microbiome composition are altered with ageing, paving the way for potential strategies to target sleep via the modulation of the gut.

Saffron (*Crocus sativus*) and its related food bioactive compounds safranal, crocin and crocetin have been reported to independently improve sleep and to affect the gut microbiota. However, the effect of saffron on sleep quality through the modulation of the microbiome is currently lacking. To address this knowledge gap, we designed a double-blind randomised placebo controlled (RCT) study (n=52, 70% Female) in older adults (65 years old) with sleep disturbance. Participants received either a placebo or a saffron extract (30 mg) for 4 weeks. Sleep quality was assessed both subjectively through validated questionnaires (Pittsburgh Sleep Quality Index (PSQI), Karolinska Sleepiness Scale (KSS), and Insomnia Severity Index (ISI), and objectively using a portable EEG device (Dreem 3). Faecal samples were collected before and after the intervention to monitor changes in microbiota composition. Dietary habits were also recorded using a food frequency questionnaire (FFQ) at the beginning of the study.

Four weeks supplementation with saffron significantly improved subjective sleep quality measures, ESS ($p<0.05$) and PSQI ($p<0.01$) scores, with better sleep efficiency observed in females ($p=0.01$). Organic sleep measurements were positively impacted with an increase in REM sleep in the saffron group in Female ($p<0.05$). Changes in sleep quality were paralleled with an increase in the genus *Faecalibacterium* and *Lachnoclostridium* ($p<0.05$) as well as a significant enrichment of the genera *Prevotella and prevotellaceae* (positive LDA Scores), which are bacteria that have previously been reported to improve circadian disturbances. In addition, *Turicibacter* was decreased in the saffron group ($p<0.05$).

These results are promising since polypharmacy is a significant problem in elderly patients. This study provides further evidence for the effectiveness of bioactive compounds improving sleep quality through gut health in older adults.

This Study was funded by UKRI-Zinc Healthy Ageing Catalyst award (ES/W006367/1).

O 050 RESEARCH ON CAROTENOIDS IN THE HEALTH-PROMOTING AND SUSTAINABLE FOODS ERA

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Carotenoids are widespread and versatile compounds. They can be cleaved enzymatically or not into a myriad of other compounds that widen considerably the actions they are involved in Nature (*1*). Although for many years their interest in food science and nutrition lied in the fact that they are natural colours and some of them are vitamin A precursors, a large body of evidence has accumulated indicating that they can contribute to reduce the risk of different diseases (cardiovascular disease, skin, eye or bone conditions, cacer, metabolic disorders) and be beneficial in relation to cognition and child development (*2*). On the other hand, the importance of carotenoids for food security is undeniable. To be aware of this it is necessary to look at the big picture of the actions carotenoids and their derivatives are involved in. Carotenoids are primordial in photosynthesis, the primary engine of food production in aquatic and terrestrial ecosystems. Furthermore, their colours and and carotenoid-derived aromas are key to attract pollinators and seed dispersers for plant propagation. Abscisic acid and strigolactones are phytohormones intervening in important processes for plant development, resilience and propagation (symbiosis with mycorrhizal fungi, resistance to abiotic stresses, seed germination, etc.). Other carotenoid-derived signals can be important at different levels for plants. Besides, carotenoids can be beneficial for the reproduction of some animals (*3–9*). It is therefore not surprising that research on carotenoids continue expanding. Indeed it is being propelled by functional and cohesive networks, such as CYTED-IBERCAROT or COST-EUROCAROTEN. In the current scenario, efforts must be directed to the obtaining of health-promoting and sustainable carotenoid-rich products. Research In this context performed in the last years by our team will be commented, including the hydro-sustainable production of tomatoes, the fine-tuning of cooking conditions to increase carotenoid bioavailability saving energy, the extraction of carotenoids from diverse matrices by sustainable techniques and solvents or the enrichment of insect biomass with carotenoids from by-products.

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O 051 LONGITUDINAL STUDY OF THE ASSOCIATION BETWEEN TOMATO CONSUMPTION AND BLOOD PRESSURE IN AN OLDER POPULATION AT HIGH CARDIOVASCULAR RISK

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Background:

Clinical studies have produced conflicting evidence on the effects of the consumption of tomatoes (*Solanum lycopersicum* L.) on blood pressure, and there are limited data from epidemiologic studies. Tomato is one of the most consumed, widely available, and affordable vegetables worldwide, and it is an important component of the best diets, such as the Mediterranean diet (MedDiet). Tomato composition includes water (95%), carbohydrates (3%), protein (1.2%), and lipids (1%), and it also contains non-sodium minerals (calcium, magnesium, phosphorus, potassium, zinc, manganese), vitamins (A, C, thiamin, riboflavin, niacin, pantothenic acid, and pyridoxine), carotenoids and phenolic compounds. Among the carotenoids, the most abundant is lycopene, which is a potent singlet oxygen quencher with antioxidant activity 10-fold higher than vitamin E. Tomato polyphenols include naringenin and caffeic, coumaric, ferulic and protocatechuic acids. Evidence from experimental and clinical studies supports the beneficial health effects of tomato, tomato-based products (e.g., sauce, juice, paste, puree, ketchup, and soup) and even lycopene taken as a supplement.

Concerning blood pressure (BP), there is conflicting evidence from clinical trials that tomato consumption may reduce systolic blood pressure (SBP), while lycopene supplementation has been reported to both reduce SBP and diastolic BP (DBP), but there is no general consensus on the effects of tomato intake on BP.

Objectives:

To assess whether tomato consumption was associated with SBP and DBP and the risk of hypertension in a prospective 3-year longitudinal study.

Methods:

A 3-year prospective longitudinal study was carried out within the PREDIMED (Prevención con Dieta Mediterránea) trial involving 7,056 (82.5% hypertensive) participants. The consumption of tomato (g/d) was estimated and categorized into 4 groups, and trained personnel measured blood pressure at the beginning, one and three years of follow-up. Multilevel linear mixed models were used to assess the association between systolic and diastolic blood pressure and tomato consumption. Hypertension risk was analyzed using Cox proportional-hazards models in 1,097 participants without hypertension at baseline.

Results:

An inverse association between tomato consumption and diastolic blood pressure was observed in comparison between the intermediate group (44–82 g/d) (β=-0.65 mmHg [95% CI:-1.20,-0.10]) and the lowest consumption group. A significant inverse association was observed for systolic and diastolic blood pressure in grade 1 hypertension participants in the intermediate tomato consumption group. The risk of hypertension decreased with consumption of >110 g/d tomato, equivalent to a medium-sized fruit (highest vs lowest consumption; HR, 0.64 [95% CI, 0.51–0.89]).

Conclusions:

The consumption of tomato and tomato-based products is associated with reductions in systolic and diastolic blood pressure, particularly in grade 1 hypertension. In non-hypertensive participants, the risk of incident hypertension was reduced by 36% in the highest consumption category. Thus, tomato consumption may play a favourable role in the prevention and management of arterial hypertension.

O 052 HUMAN INTERVENTION STUDY TO ASSESS THE URINARY EXCRETION OF ORGANOSULFUR COMPOUNDS AFTER ACUTE INTAKE OF BLACK GARLIC

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Background:

The consumption of black garlic has been related to a decreased risk of many human diseases due to the presence of phytochemicals such as organosulfur compounds (OSCs). However, information on the metabolization of these compounds in humans is limited.

Objective:

Therefore, this study aims to determine the OSCs, and their metabolites, excreted in urine 24 h after an acute intake of 20 g of black garlic by healthy humans.

Methods:

The intervention study involved 12 volunteers (7 females and 5 males), with an average age of 29 years and an average body mass index of 23.5 kg/m^2. To study the organosulfur biological metabolites a targeted chromatographic approach based on ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) was used.

Results:

A total of thirty-three OSCs were identified and quantified in urine, being the main ones methiin, isoalliin, *S*-(2-carboxypropyl)-L-cysteine and *S*-propyl-L-cysteine (deoxypropiin). Detectable amount of other metabolites were also determined including *N*-acetyl-*S*-allyl-L-cysteine (NASAC), *N*-acetyl-*S*-allyl-L-cysteine sulfoxide (NASACS) and *N*-acetyl-*S*-(2-carboxypropyl)-L-cysteine (NACPC), derived from *S*-allyl-L-cysteine (SAC), alliin and *S*-(2-carboxypropyl)-L-cysteine, respectively. These compounds appeared in urine after a potentially *N*-acetylation in the liver and kidney. The total excretion of OSCs 24 h after the ingestion of black garlic was 64,312 ± 26,584 nmol, being excreted mainly between 8–24 h after black garlic intake. This indicate that the absorption of these compounds mainly occurs in the large intestine. From these results, a tentative metabolic pathway has been proposed for OSCs in humans.

Conclusion:

The identification of these organosulfur compounds as urinary biomarkers of black garlic ingestion provides opportunities to study the role of this product in human health, and also forms the basis for the further evaluation of the biological role and health potential of these secondary plant metabolites in humans.

O 053 DETERMINATION OF BROCCOLI (*Brassica oleracea* VAR. *italica*) GLUCOSINOLATE CONTENT USING MID-INFRARED (MIR) SPECTROSCOPY COUPLED WITH CHEMOMETRICS

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Background & Objectives:

Glucosinolates are nitrogen sulphur-containing compounds found almost exclusively in *Brassicaceae* vegetables, such as Broccoli (*Brassica oleracea*), having important health and nutritional benefits. Broccoli glucosinolates are significantly affected by pre- and post-harvest practices. Thus, monitoring their content using feasible analytical methods for the food and agricultural industry is important. Conventionally chromatography techniques such as high-performance liquid chromatography coupled diode-array detection (HPLC-DAD) or liquid chromatography in tandem with mass spectrometry (LC-MS/MS) are used to identify and quantify glucosinolates in plant tissue. However, these techniques can be time-consuming, costly, and require complex pre-processing steps, which can be a great drawback when handling large sample sets. Therefore, this study evaluates the application of mid-infrared (MIR) spectroscopy as a rapid, cost effective technique for the determination of broccoli glucosinolates and differentiation between broccoli varieties.

Methods:

Freeze-dried broccoli samples (n=53) from seven varieties were analysed using MIR spectroscopy and HPLC-DAD. The infrared spectra were analysed using pre-processing methods including baseline correction and second derivative in the region of 800 to 1400 cm^{-1}. For qualitative analysis, principal component analysis (PCA) was performed to visualise the data structure and identify patterns between different varieties of broccoli and the HPLC data. Linear discriminant analysis (LDA) was used to classify the broccoli samples according to the system of production. For quantitative analysis, calibration models were developed using MIR and HPLC-DAD reference data through partial least squares (PLS) regression.

Results:

Differences in the MIR spectra of the individual broccoli varieties were observed in the carbohydrate fingerprint region (950–1100 cm^{-1}) and between 1340–1615 cm^{-1} assigned to specific glucosinolates. PCA enabled the differentiation between broccoli varieties (common vs. Tenderstem® broccoli) and was also efficient in differentiating between samples with relatively high (200–500 mg/100 g DW) and low (<200 mg/100 g DW) glucobrassicin content. LDA was also used to classify broccoli varieties according to the system of production (organic vs. non-organic) and variety (common vs. Tenderstem® broccoli). The classification rates indicated that 79% of the samples were correctly classified as organic and non-organic while 96% of the samples were correctly classified as common broccoli and Tenderstem®.

PLS calibration models were developed for the glucosinolates: glucoraphanin, glucobrassicin, neoglucobrassicn, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, glucoiberin, as well as total indole glucosinolates and total glucosinolates. Success models using all the broccoli samples, resulted in an R^2 value between 0.50 to 0.78 and an RDP value from 1.35 to 2.19. Models were also developed using samples from a single variety, Tenderstem® (organic and non-organic), which improved some of the calibration metrics (R^2 range=0.41–0.91; RDP range=0.81–2.97). These models developed using MIR spectroscopy are relatively better than those developed by near-infrared spectroscopy in the literature and could be used for screening purposes.

Conclusion:

This study demonstrates that MIR spectroscopy could be used as a potential tool to classify and monitor broccoli samples according to their variety and system of production, as well as the potential for development of calibration models to be used for screening of glucosinolates.

O 054 ALLYL-ISOTHIOCYANATE AFFECTS THE ENERGY METABOLISM OF *Drosophila melanogaster* FED A HIGH SUGAR DIET

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The incidence of type II diabetes mellitus is increasing worldwide, demonstrating the urgent need for cost-effective and easy-to-use therapies. One approach could be the use of bioactive plant compounds such as the mustard oil glycoside sinigrin and its degradation product allyl-isothiocyanate (AITC). Some cell culture studies suggest an improvement of insulin resistance by AITC treatment. However, the underlying molecular mechanisms are not fully understood. In addition, there is limited information on how AITC affects energy metabolism at the organismal level.

We used the fruit fly *Drosophila melanogaster* as a research model for type II diabetes mellitus. Feeding fruit flies a high sucrose diet (HSD) result in changes of the energy metabolism similar to those seen in the pathophysiology of type II diabetes mellitus in mammals, including the development of hyperglycemia and dyslipidemia. In the present study, *D. melanogaster* were fed either a control diet or HSD with or without AITC for 10 or 30 days, respectively. To determine possible effects of AITC on the energy metabolism of fruit flies fed HSD, glucose and triglyceride levels as well as expression levels of associated genes were measured. Oral administration of AITC in addition to HSD significantly decreased HSD-induced triglyceride levels and affected genes related to energy metabolism.

Overall, the present study suggests that AITC mediates its antidiabetic effects via an impact on the fly's energy metabolism at multiple levels, including triglyceride levels and expression levels of genes associated with the fly's energy metabolism.

O 055 ASSOCIATIONS BETWEEN NON-CHOLESTEROL STEROLS, ASTHMA SEVERITY, AND AIRWAY INFLAMMATION IN PEDIATRIC POPULATIONS: THE ADEM1 AND MIKADO STUDIES

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Background:

Asthma is a non-communicable disease affecting 262 million people globally, especially children. Asthma significantly affects quality of life and strategies to reduce asthma severity are needed. We previously found that interventions with plant stanol esters in asthmatic adults shifted *ex-vivo* immune responses away from the Th2 dominant response. We also reported in breastfed children that higher non-cholesterol sterol concentrations in breastmilk were associated with decreased prevalence of allergic outcomes in the first two years of life. Therefore, we hypothesize that serum non-cholesterol sterol concentrations also have a role in asthma prevention.

Objectives:

Associations between serum non-cholesterol sterol concentrations, asthma severity, and airway inflammation were studied in two pediatric populations (3 and 12 years old) with and without asthma.

Methods:

Data were used from the ADEM1 study (pediatric population with asthma, transient wheeze, or healthy at age 3) and from the MIKADO study (obese pediatric population with asthma or increased risk at asthma at age 12). Serum non-cholesterol sterol concentrations were measured in both studies using gas chromatography–flame ionization detection and concentrations were corrected for serum cholesterol concentrations. Both studies measured airway inflammation (fractional exhaled nitric oxide (FeNO)), and asthma severity (the ratio between flow expiratory volume in 1 second and forced vital capacity (FEV1/FVC) as marker for lung function, and quantification of inhaled corticosteroid use). Statistical analyses were performed separately for each study. For both studies, cross-sectional associations between baseline serum non-cholesterol sterol concentrations and baseline study outcomes were determined (Pearson correlations, AN(C)OVA, logistic regression). Longitudinal associations (ADEM1) and analyses of pre-post weight loss intervention changes (MIKADO) were analyzed in a similar way.

Results:

We included N=90 children from the ADEM1 study and N=41 children from the MIKADO study. For the ADEM1 study, no cross-sectional associations between serum non-cholesterol sterols and study outcomes were found. Pearson correlations between non-cholesterol sterols at baseline and outcomes at age 6 were not significant, but cholesterol absorption markers at age 3 were positively associated with FeNO at age 6 (β [95% CI]: 2.22 [0.13; 4.30]; p=0.04). For the MIKADO study, cholesterol absorption markers were negatively associated with FeNO (r=-0.64, p=0.03) and positively associated with FEV1/FVC (r=0.73, p=0.01) in the group without asthma complaints at baseline. Considering the changes in non-cholesterol sterols pre-post weight loss intervention, changes in serum cholestanol were positively associated with changes in FEV1/FVC in children without asthma complaints (r=0.66, p=0.02). Changes in serum lathosterol were positively associated with FEV1/FVC in the total population (r=0.35, p=0.02) and the asthma group (r=0.45, p=0.02). When using AN(C)OVA and logistic regression analyses instead of Pearson correlations, results were similar.

Conclusion:

The associations between non-cholesterol sterols, asthma severity, and airway inflammation seems different at the age of 3 and 12. Additionally, associations between serum non-cholesterol sterols and lung function (FEV1/FVC) seem to differ between children with and without asthma diagnosis after a weight loss intervention. The underlying mechanism of non-cholesterol sterols in asthma development and disease progression should be studied further before this dietary approach can be advised for asthma treatment and/or prevention.

O 056 POSTPRANDIAL HYPERLIPIDEMIA MODULATES HIGH DENSITY LIPOPROTEIN CHOLESTEROL EFFLUX CAPACITY IN A FATTY ACID-DEPENDENT MANNER

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Background:

High density lipoprotein (HDL) main function is the transport of cholesterol from peripheral tissues to the liver, and HDL-associated cholesterol (HDL-C) has been traditionally considered as a protective factor against cardiovascular diseases (CVDs). However, recent evidence suggests that HDL functionality is modulated by different factors, which could turn into pro-atherogenic and dysfunctional particles.

Objectives:

The aim of this study was to evaluate the effects of dietary fatty acids in HDL-associated cholesterol efflux capacity during the postprandial metabolism.

Methods:

Healthy male volunteers were recruited for an interventional study with high fatty acid-based meals, based in emulsions enriched in ω3-long chain polyunsaturated fatty acids (ω3- LCPUFAs), monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), or no-fat emulsion. Blood samples were collected at basal time and 2–3 hours and 5–6 hours after the meal intake and serum was obtained and stored until analysis. Cholesterol efflux capacity (CEC) was analyzed using apoB-depleted serum and fluorescent labelled cells.

Results:

During the postprandial time, HDL-associated CEC was modified. Fat rich meals produced an increase of CEC, and no fat meals produced a reduction of CEC. In specific manner, ω3-LCPUFAs- (mainly docosahexaenoic and eicosapentaenoic acids) and MUFAs- (oleic acid) enriched meals produced a higher increase in HDL-associated CEC compared to SFAs (palmitic and stearic acids)-enriched meals.

Conclusion:

HDL-associated CEC is modified during the postprandial metabolism in a fatty-acid dependent manner.

O 057 A PROBIOTIC FUNCTIONAL FOOD ENRICHED IN PHYTOSTEROLS AND CAROTENOIDS TO TARGET HYPERCHOLESTEROLEMIA, INSULIN RESISTANCE, VITAMIN A STATUS AND GUT MICROBIOTA IN HIGH FAT DIET-INDUCED METABOLIC SYNDROME RATS

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Tackling Metabolic Syndrome (MetS) by functional foods particularly fermented food have recently attracted more attention[1]. A vegetable fermented maize and fruits-based food was designed to be functional namely probiotic and enriched in papaya/melon carotenoids and dispersible phytosterols in order to obtain a cholesterol-lowering effect without deleterious effect on blood carotenoid level[2]. The aim of this work was to investigate the effect of this new functional food on a HFD (High-Fat-Diet)-induced MetS rat model focusing on dyslipidemia insulin-resistance, taking into account vitamin A status and gut microbiota.

An amount of 1 g/rat/day of the fermented corn (5%) and fruit (30%) diet was administered to the rats and contains 42 µg of carotenoids and 18 mg of phytosterols equivalent to the dose of 1, 6 to 2 g per day necessary to obtain a hypocholesterolemic effect in humans. Male Sprague-Dawley rats (n=36) were divided into 4 groups of 9 rats : a control group (C), an overweight and prediabetic group (HFD diet), an HFD-P preventive group receiving in addition the functional food during the 3 months and a curative group HFD-CU receiving the food from the 2nd month. After enthanasia (isoflurane 4 %), blood, liver, jejunum and ileum were collected.

The functional food exerts significant anti-Mets/T2D effects by reducing LDL cholesterol and tryglycerides (1.5 times compared to the HFD group for HFD-CU and HFD-P- $p<0.05$). Fasting insulin and HOMA-IR index indicate that the supplementation both in preventive and curative groups ameliorated HFD-induced insulin resistance by 2,5 to 3 fold compared to the HFD group $p<0,005$). Interestingly, the vitamin A status in liver of HFD rats was significantly ($p<0.05$) lower than all other groups (278 nmol/g liver vs 425, 357 and 350 respectively in Control, HFD-CU, HFD-P). The rats treated with functional food showed a similar vitamin A status compared to the control group, indicating an effective restoration of vitamin A status in MetS rats. Additionally, the two treatments significantly downregulated the hepatic mRNA expressions of SOD, Glu-Px (oxidative stress), IL6, GmCsf , Ikba (inflammation), PPARg (lipid metabolism), and increased chREBP and CD 206 expression.

Gut microbiota (GM) was analysed using 16S rRNA high-throughput sequencing. At family level, HFD increased *Morganellaceae* (a member of *Enterobacteriales* order), *Desulfovibrionaceae* (a Proteobacteria) and *Prevotellaceae*. HFD decreased *Eubacterium coprostaligenes* group (a cholesterol-reducing bacteria), *Muribaculae* (a Bacteroidota member) and other minority families. The GM was modified by the two HFD treatments. Globally, the preventive treatment modified more GM groups than that of the curative. Interesting, the abundance of *Ruminococcaceae*, a family playing an important role in preventing metabolic syndrome, was increased with the two treatments.

Together our results suggest that lipid and glucid metabolism as well as vitamin A status were improved regulating gut microbiota by consumption of the functional fermented food. The specific formulation of this fermented product could be used for the prévention and management of MetS associated to obesity and T2D.

1. Jalili, M., M. Nazari, et al. (2023). «Fermented Foods in the Management of Obesity: Mechanisms of Action and Future Challenges.» Int J Mol Sci **24**(3).

O 058 MASTIHA OIL EXHIBITS FAVORABLE EFFECTS IN METABOLICALLY UNHEALTHY ADULTS – A RANDOMIZED CONTROLLED TRIAL

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Mastiha oil is the essential oil that comes out through steam distillation of the resinous exudate of *Pistacia lentiscus* of Chios. It is rich in monoterpenes α-pinene, β-myrcene, β-pinene. Until now it has been used as a food additive, particularly as a flavouring agent, in cooking and pastry. Its constituents exhibit antioxidant and anti-inflammatory activities. In the present 3-month randomized controlled trial, we investigated the effects of Mastiha oil on individuals with abdominal obesity and metabolic abnormalities i.e., dyslipidemia, hypertension, insulin resistance. Eligible patients (N=94) were randomly assigned to either the intervention group, receiving capsules containing 200 mg of Mastiha oil daily for 3 months, or the control group. Anthropometric measurements, blood markers and health-related quality of life (QoL) were assessed. Statistical analysis was performed on an intention-to-treat basis. Results showed a significant improvement in blood lipid profile, namely triglycerides ($p=0.026$) and low-density lipoprotein ($p=0.05$) of the Mastiha oil group versus controls. Systolic blood pressure ($p=0.05$) and alanine aminotransferase ($p=0.022$) significantly decreased at 3 months only after Mastiha oil intake. Body weight decreased only in the Mastiha oil group ($p=0.02$), while mean changes in % body fat ($p=0.005$) and visceral fat level ($p=0.045$) were significantly different between arms. Lower oxidized LDL ($p=0.044$) and greater adiponectin ($p=0.007$) were recorded in the Mastiha oil group with the mean changes being significant different between arms. Finally, QoL, as assessed by Short Form-12 questionnaire was improved in the Mastiha oil group compared to control ($p=0.041$ for Physical Composite Score, $p=0.035$ for Mental Composite Score). No adverse effects were reported. An anti-obesity effect of Mastiha oil is suggested, probably due to modulation of inflammatory and antioxidant processes, as well as adipocyte function. In conclusion, this Mediterranean food additive is effective in regulating parameters associated with metabolic abnormalities.

O 059 SOURCE-SPECIFIC NITRATE INTAKE AND INCIDENT DEMENTIA IN THE DANISH DIET CANCER AND HEALTH STUDY COHORT

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Background:

Dietary nitrate, through its conversion to nitric oxide – a pleiotropic signalling molecule that plays a fundamental role in the cerebrovascular and central nervous systems – is hypothesised to lower the risk of dementia. However, dietary nitrate also has the potential to form *N*-nitrosamines, which are potentially neurodegenerative. The dietary source of nitrate, whether that be plant-derived foods, animal-derived foods, or water, is hypothesised to govern the fate of nitrate in the body. Whether dietary nitrate from different sources differentially associates with incident dementia is yet to be investigated.

Objectives:

Our primary objective was to examine prospective associations between dietary nitrate intake, from different sources (namely, plant, animal, and water sources) and incident dementia.

Methods:

In ~54,800 participants of in the Danish Diet Cancer and Health cohort, between 50 and 65 years and who were not registered as having dementia at baseline (1993–1997), we examined associations between intakes of 1) plant-sourced nitrate, 2) animal-sourced nitrate and 3) water-sourced nitrate, and incident dementia. Nitrate intake from the different dietary sources was estimated from a food frequency questionnaire, completed by participants at baseline, using databases on the nitrate content of 1) plant foods, 2) animal foods, and 3) water supplies, obtained by linkage with participant address/es in the 12 months prior to baseline. Incident dementia was defined as a record of a hospital contact with dementia diagnosis or the collection of a prescribed medication for dementia, captured in the Danish Register for Selected Chronic Diseases until 2020. Associations between source-dependent nitrate intake and incident dementia were examined using restricted cubic splines within Cox proportional hazards models, adjusted for demographic, lifestyle, and dietary confounders.

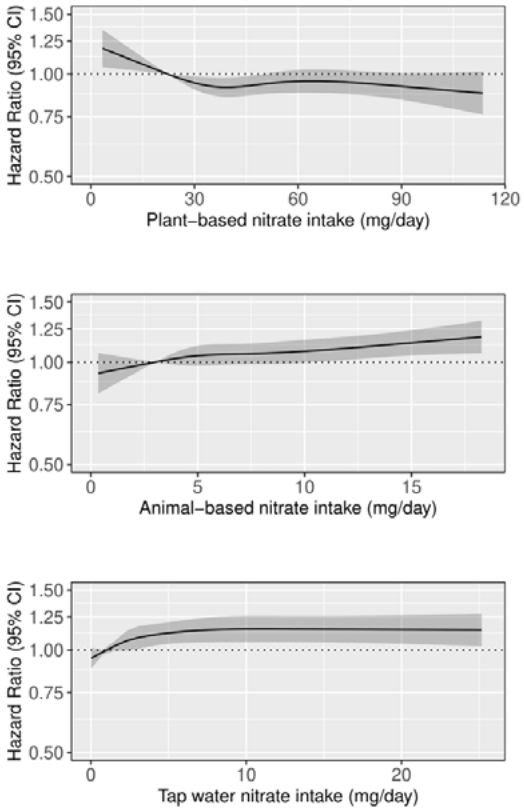
Results:

Over ~25 years of follow-up, ~4,700 participants met the criteria for incident dementia. In multivariable-adjusted models, higher intakes of plant-derived nitrate, of which vegetables contributed ~80%, were non-linearly associated with a lower risk of incident dementia, while higher intakes of animal-derived nitrate and water nitrate were non-linearly associated with a higher risk of incident dementia (**Figure 1**). Participants with the highest intakes (quintile 5) of plant-derived nitrate had a 10% [HR 95% CI: 0.90 (0.83, 0.98)] lower risk of dementia, while participants with the highest intakes of animal-derived nitrate and water nitrate had a 14% [HR 95% CI: 1.14 (1.05, 1.23)] and a 17% [HR 95% CI: 1.17 (1.08, 1.28)] higher risk of dementia, respectively, compared to their low nitrate consuming counterparts (quintile 1).

Conclusion:

Dietary nitrate may modulate dementia risk in a manner that is contingent upon dietary source. Encouraging higher consumption of plant-derived nitrate and lower consumption of nitrate from non-plant sources may help to reduce dementia incidence. These findings warrant further investigation to aid in the development of dementia prevention strategies.

Figure 1. Cubic spline curves depicting associations between 1) plant-sourced nitrate, 2) animal-sourced nitrate, and 3) water nitrate and incident dementia in the Danish Diet Cancer and Health Study cohort. Hazard ratios are based on Cox proportional hazards models adjusted for age, sex, BMI, smoking, physical activity, education, living situation, and alcohol intake (g/d).



O 060 POTENTIAL OF OAT-DERIVED COMPONENTS IN THE PREVENTION OF HYPERTENSION AND ITS CARDIOVASCULAR COMPLICATIONS

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Background:

Hypertension is one of the major risk factors for developing cardiovascular disease, the number one cause of death and disability in most parts of the world.

Objectives:

Our goal was to assess the potential of oat derived components in preventing blood pressure elevation and providing cardioprotection in two preclinical studies.

Methods:

In the first study we examined the effects of oat-derived avenanthramide C and beta-glucan alone, or in combination, in an animal model of primary hypertension, the spontaneously hypertensive rats (SHR). Five-week-old male SHR and their age-matched normotensive controls, Wistar-Kyoto rats (WKY), received vehicle, avenanthramide C and beta-glucan alone or in combination of avenanthramide C and beta-glucan via oral gavage daily for 15 weeks. Blood pressure (BP) and echocardiography measurements were performed on all animals. Markers of oxidative stress, inflammation and vascular function were also assessed in blood samples from all animals. In the second study we are currently examining the cardiovascular effects of oat beta glucan in male vs. female SHR, as well as the comparative and combinatorial effects of oat beta glucan and a standard antihypertensive medication, hydrochlorothiazide in these animals.

Results:

The results of the first study showed that SHR had very high BP and a prolongation of isovolumetric relaxation time (a parameter assessing diastolic heart function). Administration of beta-glucan alone prevented the elevation of BP in SHR (when compared to SHR treated with vehicle), however avenanthramide C alone or the combination were not effective. Similarly, SHRs administered with beta-glucan and not avenanthramide C or the combination prevented the prolongation of isovolumetric relaxation time (in comparison to SHR treated with vehicle). Administration of beta-glucan alone and avenanthramide C alone also prevented the increase in oxidative stress in SHR (when compared to SHR treated with vehicle). The results of the second study which is currently ongoing will also be presented and discussed in the presentation.

Conclusions:

The results of our preclinical studies thus far suggest that beta glucan may have antihypertensive and cardioprotective benefits. The beneficial effects of beta-glucan may in part be due to preventing oxidative stress.

O 061 VALORIZATION OF ENDIVE CO-PRODUCTS FOR FUNCTIONAL FOOD APPLICATIONS

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Background:

Endive (*Cichorium intybus* L. var. *foliosum)* is produced in Netherlands, Belgium and France, the world's largest producer, with 160 000 tones per year. This Asteracea is rich in inulin, a polymer of fructose, well-known for different health properties like anti-diabetic, hepato-protective or prebiotic. Endive is also rich in specialized metabolites like polyphenols which are effective antioxidants and were also described for antimicrobial, anti-hyperglycemic and anti-cancer activities. However, the endive production process generates many co-products that are not fully valorized and still contain inulin and/or polyphenols (Perović et al. 2021).

Objectives:

The study aims to improve antioxidant, hepato-protective and anti-inflammatory activities thanks to the bio-conversion of endive by-products. Different ways exist to convert co-products for valorization as methanation, silage, extraction, fermentation or enzymatic treatments.

Method:

We first measure the antioxidant activity of endive co-products after bio-conversion with the radical scavenging DPPH test, widely used for food applications (Le Rouzic et al. 2022) . Then, the effect of converted co-products on ROS production with 2',7'-dichlorodihydrofluorescein diacetate method was measured in hepatic and intestinal cell lines. Furthermore, we developed an innovative cell-based assay to evaluate the hepato-protective potential of the samples. Finally, the anti-inflammatory activity of endive co-products was assessed in macrophages by measuring cytokine secretion with ELISA assays.

Results:

First results suggests that endive by-products are more bioactive after bio-conversion. This was marked for antioxidant activity.

Conclusion:

The bio-conversion is an efficient and sustainable way of valorization. It leads to improve bioactivities that are beneficial for human health. The final products have good potential to be used in several applications intended for cosmetics, pet food and the food industry.

Keywords:

Endive co-product, valorization, antioxidant activities, hepato-protective activity, anti-inflammatory activity, bio-conversion

References:

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O 062 FIBRE AND PREBIOTIC SUBSTANCES FROM STARCH IN HUMAN NUTRITION

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Obesity and overweight, which are significant social problem, affect up to 20% of the developmental population and 50% of adults. The main factors contributing to the development of obesity include excessive consumption of products containing easily absorbable, high-calorie nutrients, including simple sugars. The development of obesity is also closely correlated with changes in the intestinal microbiota. Eating a diet high in dietary fibre and prebiotics is essential in the prevention and treatment of obesity and its complications. Oligosaccharides, mainly fructooligosaccharides (FOS), are commonly used as soluble dietary fibre with prebiotic properties. The main limitation of their use is that they cause gastric problems. Moreover, they are poorly tolerated by many people, especially with irritable bowel syndrome (IBS). The use of starch products, such as resistant dextrins, resistant maltodextrins and soluble corn fibre gives great opportunities in this regard. The results of the research obtained as part of the project with the acronym PreSTFibre4kids are presented. This project is aimed at examining vegetable and fruit mousses with the addition of soluble dextrin fibre (SDexF) from potato starch with prebiotic properties, in terms of the prevention of overweight and obesity in children and the reduction of metabolic disorders secondary to obesity. In the first stage, an innovative method of obtaining SDexF on a semi-industrial scale was developed. Then, SDexF was subjected to comprehensive physico-chemical characterization and nutritional labeling. Based on analysis, including assessment of the composition and nutritional value, as well as safety assessment, National Institute of Public Health issued a positive recommendation recognizing SDexF as a food ingredient. In the next stage, industrial partner developed recipes of 6 flavors of vegetable and fruit mousses with and without addition of SDexF. The organoleptic characteristics of mousses were assessed using acceptance and preference methods according to the criteria developed in The Children's Memorial Health Institute. The most accepted and preferred: apple-carrot-quince, apple-peach-parsnip-lemon, apple-cherry-carrot-banana mousses were selected for further clinical trials. The study was performed in a group of 100 children aged 6 to 10 years, using a double-blind procedure. Evaluation points were anthropometric, metabolic, immunological parameters and changes in intestinal microbiota and metagenome.

Acknowledgements:

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O 063 EXPLORING THE EFFECT OF A MULTI-SPECIES PROBIOTIC ON COGNITIVE FUNCTION AND MOOD IN HEALTHY OLDER ADULTS, AND AN EXPLORATION INTO MICROBIALLY-DERIVED METABOLITES AS A MECHANISM OF ACTION

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Decline in cognitive functions such as memory and executive function are hallmarks of ageing, even in the absence of age-related disorders. Additionally, increased vulnerability to mental health disorders such as depression is common in older adults. Probiotic supplements are rapidly gaining interest as functional foods for improving cognitive and psychological function, via the gut-brain axis. As such, the present study employed a randomised, placebo-controlled cross-over trial in 30 healthy older adults to explore both the acute (1 day) and chronic (8 weeks) effects of a multi-species probiotic supplement (Ecologic Barrier) on various cognitive domains of verbal memory and learning, spatial working memory and executive functions, alongside mood measures such as perceived stress, anxiety, depression, and cognitive reactivity to sad mood. 16s rRNA sequencing of stool samples was also performed pre- and post-intervention to assess potential effects on the gut microbiota community. Chronic probiotic supplementation was associated with the attenuation of poorer executive function during higher cognitive demand, and improvement in cognitive biases such as hopelessness, rumination and aggression that contribute to reactivity to sad mood and therefore vulnerability to depression. Novel acute probiotic supplementation was associated with significantly faster reaction times during a task of executive function.

Alongside the RCT, *in vitro* continuous three-stage culture models were run in triplicate with a view the exploring microbially-derived metabolites as a potential underlying mechanism of action. Short-chain fatty acids (Gas Chromatography), bacterial synthesis of various neurotransmitters (Liquid Chromatography Mass Spectroscopy), and microbial community (Flow-FISH and 16s rRNA sequencing) were assessed at steady state and following daily addition of Ecologic Barrier. A consistent significant increase in the relative abundance of the *Lactococcus* genus was found in both the *in vitro* models and following chronic supplementation in the RCT. Trends in the *in vitro* data suggest that probiotics may enhance the production of SCFAs and GABA, but the current work provides no strong evidence for an effect on neuroactive metabolites and highlights that bacterial derivation is likely not a primary production pathway for neurotransmitters other than GABA under physiologically relevant conditions.

As such, the current work provides further support for improved cognitive reactivity to sad mood following supplementation with Ecologic Barrier and some limited support for improved executive function in a healthy ageing population, but microbially-derived neurotransmitters are perhaps unlikely to provide a key underlying mechanism of action.

O 064 DEVELOPMENT OF A PLATFORM FOR THE ANALYSIS OF BIOMARKERS OF FOOD INTAKE WITHIN THE EU H2020 PREVENTOMICS PROJECT

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Background:

Metabolomics allows the identification of biomarkers of food intake (BFIs), ideally more objective measures of exposure than self-reported methods. Accurate measurement of food consumption is crucial to understand the link between diet and the risk of development of diet-related diseases. The EU H2020 PREVENTOMICS project has developed personalized plans for nutrition and lifestyle habits to improve individuals' health.

Objective:

This work aimed at developing a validated analytical platform for the analysis of BFIs that allows an actual assessment of the diet of European consumers.

Methods:

A list of candidate biomarkers reflecting the intake of specific food groups and foodstuffs was established based on comprehensive literature searches and after matching a series of eligibility criteria. An analytical LC-MS/MS method to measure candidate BFIs in urine samples was developed and quantification was performed with calibration curves of analytical standards, when available. In parallel, a comprehensive Excel-based database that links BFIs with individual foodstuffs and food groups consumed by the European population was developed. This allowed the comparison between the information collected through self-reported methods (3-day dietary records) and the information provided by targeted metabolomics as, to date, it represents the best strategy to validate the developed methodological approach. The validation of the candidate BFIs was carried out by Spearman's rank correlations, to verify the biomarker capability to change with variations in the amount of foodstuffs or food categories consumed, and by linear regression models, to assess the predictive capability of the model developed for each biomarker.

Results:

Starting from more than 400 potential biomarkers, 195 compounds were selected for the final list of candidate BFIs. Certain biomarkers were highly specific for specific food items (as phloretin-2'-glucuronide for apple), while others were mainly reflecting the intake of specific food groups (as proline betaine for citrus fruits) or general food categories (as sulfate conjugates of 5-(3',4'-dihydroxyphenyl)-γ-valerolactone for plant-based foods). The assessment of the dietary patterns of the consumers in the European general population was carried out by pooling the data sets of previous studies and the data collected from the four PREVENTOMICS trials at baseline. Food intake biomarkers reached different levels and presented diverse ranges in urine samples of European individuals. Moreover, the data collected allowed us to assess the consumption of single foodstuffs and food categories for each dietary intervention before and at the end of the intervention period. Lastly, data supported objective observations on the effect of the intervention and on study compliance at both population and individual levels. Some significant differences were observed for BFIs before and after the PREVENTOMICS intervention in the four trials and between control and treated volunteers after the study. Most of the biomarkers presented weak to moderate significant correlations with the intake of their associated food items/food categories, in line with the available literature.

Conclusions:

The platform developed has allowed the analysis of BFIs in urine and the discovery of food items consumed by human volunteers in different intervention trials, using a validated approach for the European population.

O 065 SYSTEMATIC LITERATURE-BASED VALIDATION OF BIOMARKERS OF FOOD INTAKE FOR MULTIPLE PLANT FOODS

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Biomarkers for food intake (BFI) can serve as a valuable and objective tool to complement traditional dietary assessment methods in epidemiological studies related to nutrition and health. In recent years, numerous promising biomarker candidates have been identified through metabolomics studies. Within the FoodBAll project BFIs for many foods have been compiled in systematic reviews [1] and validated according to the validation criteria developed by Dragsted et al. [2].

The current JPI HDHL project, "Food Phytochemicals Matter for Cardiometabolic Health" (FoodPhyt), is focused on validating BFIs. One goal within the FoodPhyt consortium is to collect and careful validate BFI candidates for plant or plant-based foods that are most commonly consumed in Europe. These have been selected based on various dietary surveys conducted in different countries across the EU. The corresponding BFIs for vegetables (Lettuce (Lactuca spp.), Tomato, Cucumber, Zucchini, Peas, Brassicaceae (Broccoli, Cauliflower, Cabbage), Spinach, Green Beans, Champignon, Onion, Carrot, Asparagus, Celery, Potatoes), fruits (Strawberry, Bilberry, Orange, Apple, Banana, Grapes, Peach, Pear, Kiwi) and other foods (Walnut, Olive oil, Cocoa, Whole grain (Wheat, Rye, Oat, Barley), Coffee, Black Tea, Wine (red)) were summarized. For foods not being covered within the FoodBAll reviews, we conducted an analogous systematic literature search to collect the BFI candidates. In total, 188 publications were included, resulting in 570 marker candidates that have been applied to the validation process. Based on the eight validation criteria proposed by Dragsted et al. [2], a scoring system combined with a decision tree was developed. This enables an objective evaluation of BFI by considering its specificity, plausibility, time- and dose-response, robustness, reliability, repeatability, and analytical performance. Furthermore, the BFIs were classified into different categories such as short- and long-time marker or based on the degree of specificity as single food or food group markers. For example, alkylresorcinols (AR) are group-specific intake markers for whole grains, particularly rye and wheat, and in lower concentrations in barley. Avenanthramides, on the other hand, are specific markers for oat intake. AR only serve as markers in blood, in urine only metabolites can be detected. Some metabolites, such as 5-(3,5-dihydroxyphenyl)-pentanoic acid, remain specific for whole grain, while others like 3,5-dihydroxybenzoic acid (DHBA) and 3-(3,5-dihydroxyphenyl)-1-propanoic acid (DHPPA) are not specific. DHPPA is a metabolite of sinapic acid and flavonoids, which are present in many foods. DBHA is present in peanuts and hill raspberries [3]. This example clearly demonstrates the importance of comprehensive and careful validation of a BFI candidate before its application.

This work aims to estimate the applicability of the BFI for the selected plant foods using a comprehensive data set. The systematic approach also enabled us to identify missing data and provide recommendations for future studies.

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O 067 MULTIMETHOD APPROACH TO DETERMINE DIETARY BIOMARKERS IN HUMAN URINE USING LC-MS/MS ANALYSIS SIMULATING CONDITIONS CLOSE TO EVERYDAY LIFE

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The evaluation of the nutritional status is often difficult due to different sources of bias. Many of these biases occur while assessing the own diet with commonly used methods like food diaries or food frequency questionnaires. An alternative method to evaluate the nutritional status is the use of biomarkers, which supposed to have a higher accuracy then traditional data collecting methods. Biomarkers are substances or their metabolites which are ingested through food and later excreted in urine, which can be detected by LC-MS/MS measurement. The use of multimethods allows to analyze dietary biomarkers from several different foods with a lower analytical effort and therefore enables to assess the nutritional status more easily. Even though, two separate LC-MS/MS methods are required due to huge differences between the polarity of the chosen biomarkers. Furthermore, several sample preparation methods are used to enable the quantification of glucuronidated and sulfated metabolites.

One of the more commonly used study designs to evaluate the suitability of different biomarkers and to assess the eating habits is the intervention study. First of all, a wash out period is conducted, afterwards, one or more meals containing a higher amount of the certain food are eaten followed by collecting urine samples over several hours. These methods are often suitable to evaluate the relationship between the intake of certain foods and urinary concentration of the corresponding biomarkers and to determine the kinetics. However, one disadvantage using this kind of study design is that it enforces differences to the normal eating habit. Therefore, a study was conducted to evaluate the suitability under more realistic circumstances. Especially, the wash out period was left out and size and composition of the served meals was closer to everyday food. It turns out, that it was no longer possible, to distinguish the concentration of several biomarkers from the blank urine samples with more realistic nutrition even though, these have previously been shown to be suitable in interventions studies. Especially the distinction between the level of analyte in the blank urine and the intake of smaller amounts of the certain food due to the missing wash out period seem to be the obstacles.

O 068 A SYSTEMATIC REVIEW OF FACTORS AFFECTING THE INTER-INDIVIDUAL VARIABILITY IN THE PRODUCTION AND BIOAVAILABILITY OF (POLY)PHENOLIC METABOLITES

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Divergent responses have led to controversial results in clinical trials administering (poly)phenols, implying that the "one-size-fit-all" approach is not adequate to explore their potential protective effects. Differences in the response could be linked to inter-individual variability (IIV) in (poly)phenol bioavailability and metabolism, and determinants such as age, sex, dietary and lifestyle habits, gut microbiota composition, and genetic background. An overview of the available scientific evidence on IIV in phenolic metabolite production and bioavailability and their driving factors was obtained by systematically reviewing the literature. Literature searches were performed in Scopus and Web of Science. One hundred forty-four human studies met the inclusion criteria to assess the differences in the metabolism and absorption of phenolic compounds and their determinants for all the different sub-classes. Two types of IIV in the bioavailability of (poly) phenols were observed: one resulting in qualitative metabotypes (*selective production*) discriminated by the capability of production or not of specific metabolites and mostly related to gut microbiota composition and activity, as seen for isoflavones, ellagitannins, and resveratrol, and another one resulting in quali-quantitative/quantitative metabotypes (*continuous production*) characterized by gradients in the level of production of metabolites as observed for flavan-3-ols, lignans, prenylflavonoids, flavanones, and most phenolic sub-classes. The specific determinants of IIV are almost clear only for isoflavones and ellagitannins, while for other sub-classes they are not sufficiently characterized yet. Phenolic metabotypes are usually assessed in relation to individual phenolic sub-classes and specific metabolites and do not consider the whole spectrum of phenolic metabolites, hindering a broader observation of different patterns of (poly)phenols metabolism and bioavailability. Future studies should be designed in order to elucidate the influence of the IIV determinants on a whole set of phenolic metabolites and catabolites and to define broad (poly)phenolic metabotypes, that will might improve the assessment of specific association between (poly)phenols and health-related outcomes.

O 069 FLAVONOIDS AND THEIR METABOLITES AS POTENTIAL SOURCES OF NOVEL ANTIPLATELET DRUGS

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Background:

Epidemiological studies implied that higher intake of flavonoids from food is associated with lower incidence of cardiovascular diseases including ischemic stroke. Possible explanation of this phenomenon can be based on antiplatelet activity of flavonoids or their metabolites.

Objectives:

To test if parent (iso)flavonoids and their known metabolites can at biologically achievable concentrations affect platelet aggregation, 2) to confirm the results both in healthy persons and patients, 3) to assess the mechanism of action, and 4) to investigate structure-activity relationship

Methods:

Series of (iso)flavonoids, their known colonic metabolites and chemically related derivatives were incubated with whole human blood or platelet rich plasma. Platelet activity was tested *ex vivo* by different methods (standard turbidimetry, impedance aggregometry, ELISA detection of prostanoids). Groups of 50 generally healthy persons as well as 15 patients suffering from familial hypercholesterolemia and 50 diabetes type I patients were enrolled for confirmation of results.

Results:

Isoflavonoids were in general more potent than flavonoids with tectorigenin reaching even the activity of the standard antiplatelet drug, acetylsalicylic acid (ASA). In contrast to ASA, it acted as an antagonist at thromboxane receptors. Other isoflavonoids daidzein and genistein had dual mechanism of action involving mentioned antagonism as well as inhibition of platelet cyclooxygenase 1. Only 4 out of 29 metabolites appeared to have a clinically relevant effect. 4-methylcatechol (4MC) was the most active of them by far. It inhibited platelet aggregation with an IC_{50} of approximately 3 μM, which is about one order of magnitude lower than that of ASA. These results were confirmed in groups of generally healthy persons as well as in patients. Future mechanistic investigation revealed an uncommon mechanism of action; 4MC did not block cyclooxygenase 1 or thromboxane synthase at reasonable concentrations but affected coupling of these reactions. Structure analysis uncovered that catecholic ring is not needed for a strong antiplatelet effect.

Conclusion:

4MC, a common metabolite of many flavonoids, is a potent antiplatelet compound with higher potency than ASA. Further *in vivo* investigation of 4MC or some of its structural congeners can lead to novel antiplatelet drugs.

Conflict of interest:

None.

Acknowledgement:

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O 070 NEW ANALYTICAL CHALLENGES TO EVALUATE (POLY)PHENOL METABOLISM AND BIOACTIVITY

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In the last decades, researchers collected substantial evidence regarding the metabolism and bioavailability of (poly)phenols, intensely debating the role of human and gut microbiota enzymatic activity in (poly)phenol biotransformation, and resulting in the identification of a plethora of potentially bioactive metabolites and catabolites. Collecting biological fluids, such as plasma, urine, and faeces, and applying innovative targeted and untargeted mass spectrometry analysis were fundamental to identifying newly formed metabolites derived from (poly)phenol ingestion. Despite the increasing evidence that (poly)phenol daily consumption results in several non-communicable chronic disease prevention, a few pieces of evidence are available on the ability of tissues and organs to absorb and eventually further metabolize the circulating bioactive metabolites and catabolites. Moreover, their *in situ* distribution in target tissues and organs, where bioactive compounds would exert their biological activity, is almost unknown.

Mass spectrometry imaging (MSI) has emerged in the last decades as a powerful methodology for understanding complex biological systems. MSI application results in a simultaneous spatial distribution map of a wide range of compounds. This analytical technique has been primarily applied in drug research, to shed light on the spatio-temporal aspects of biology, and clinically relevant areas, to provide better diagnoses and prognoses and assess disease treatment.

Therefore, MSI would represent a new challenge to elucidate the spatial *in vivo* (poly)phenol metabolism, absorption and body distribution of potentially bioactive metabolites and catabolites derived from (poly)phenols. Detailed analysis of the distribution of (poly)phenol bioactive metabolites and catabolites within target tissues and organs would be a sensible way forward in the science related to (poly) phenol bioactivity, appearing as an innovative possibility to explore the local metabolism and distribution of (poly)phenols and their preferred site of accumulation and action.

O 071 HEALTH BENEFITS OF DIETARY POLYPHENOLS THAT ARE DRIVEN BY THEIR INTERACTIONS THE GUT MICROBIOTA

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There are numerous epidemiological studies and dietary intervention studies that provide evidence that consumption of certain dietary polyphenols is associated with decreased risk of certain diseases and can cause beneficial changes in biomarkers associated with disease risk and progression. Even for the most bioavailable polyphenols such as (-)-epicatechin, only a fraction of the ingested dose is absorbed from the upper gastrointestinal tract, and most of a dose of ingested polyphenols reaches the colon where they interact with the gut microbiota. There are numerous examples that have emerged over the last few years that demonstrate how important some of these interactions are.

In this talk, several examples of important interactions between polyphenols and the gut microbiota will be presented. These will include examples of the gut microbiota-dependent transformation of ingested polyphenols into new structures which are absorbed and appear to be the active forms that cause a health benefit. There are also examples where there are inter-individual differences in the microbiota-dependent metabolites that are generated and health benefits are only observed in some 'metabotypes'. There are also numerous examples of polyphenols and polyphenol-rich extracts altering the structure and diversity of the gut microbiota and altering the abundance of specific species associated with a healthy gut phenotype. Polyphenols have also been shown to change some important metabolic processes mediated by the gut microbiota, including the production of short chain fatty acids and the modification of bile acids. There is also evidence that specific polyphenols can inhibit harmful microbial metabolic processes such as the generation of the atherogenic precursor trimethylamine (TMA) from choline and L-carnitine; TMA is converted to TMA-N-oxide (TMAO) in the host liver and TMAO is strongly associated cardiovascular disease risk and has shown to cause platelet aggregation and vascular inflammation.

O 072 GUT IT ON: HOW MICROBIAL (POLY)PHENOLS METABOLITES KEEP YOUR BRAIN INFLAMMATION-FREE

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Neurodegeneration reside on multifactorial changes with complex mechanisms and no existing cure. Nevertheless, evidences suggests that neuroinflammation is a key mechanism on the development of neurodegenerative diseases. Prevention and treatment will require multi-targeted therapeutics with a focus on their anti-inflammatory properties. Studies with polyphenols have proven their pleiotropic ability to modulate several cellular pathways important on inflammation and disease. However, for a huge number of polyphenol metabolites originated from our diet, the low molecular weight polyphenol metabolites, much is still unknown. Absorption and blood concentrations of some of these low molecular weight polyphenol metabolites reach high blood concentrations and studies have shown their ability reach the brain. Yet, our understanding of their effects is still quite low.

We have been working on the ability of several low molecular weight polyphenol metabolites, present in human circulation upon dietary interventions to modulate neuroinflammation in microglia. We have tested more than seventy low molecular weight polyphenol metabolites ability to impair TNFα release by microglia cells upon an inflammatory stimulus. Several concentrations were evaluated in order to conclude which molecules showed a higher impact on the modulation of inflammation. Meanwhile the mechanisms by which these molecules can alter TNFα release by microglia cells are being elucidated, in this respect we conducted a Proteome and Kinome study and demonstrated the impact of these molecules on several inflammatory pathways such as TGF, NFκB, MAPK and Jack-Stat We are currently validating these findings by analysing inflammatory markers on the mouse brain, on a animal model of Parkinson's disease.

In conclusion, we are deciphering the role of low molecular weight polyphenol metabolites at physiological conditions, exploring the mechanism for their anti-inflammatory properties in microglia cells and validating this on a Parkinson's disease animal model. Altogether we hope to understand how these molecules could potentially be a useful tool to modulate neuroinflammation and limit the process of neurodegeneration.

Acknowledgments:

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O 073 CRANBERRY PROCYANIDINS AID IN MAINTAINING A HEALTHY GUT BY PROMOTING A FAVORABLE GUT MUCOSAL ENVIRONMENT AND A UNIQUE INTERACTION BETWEEN THE MICROBIOTA AND THE HOST

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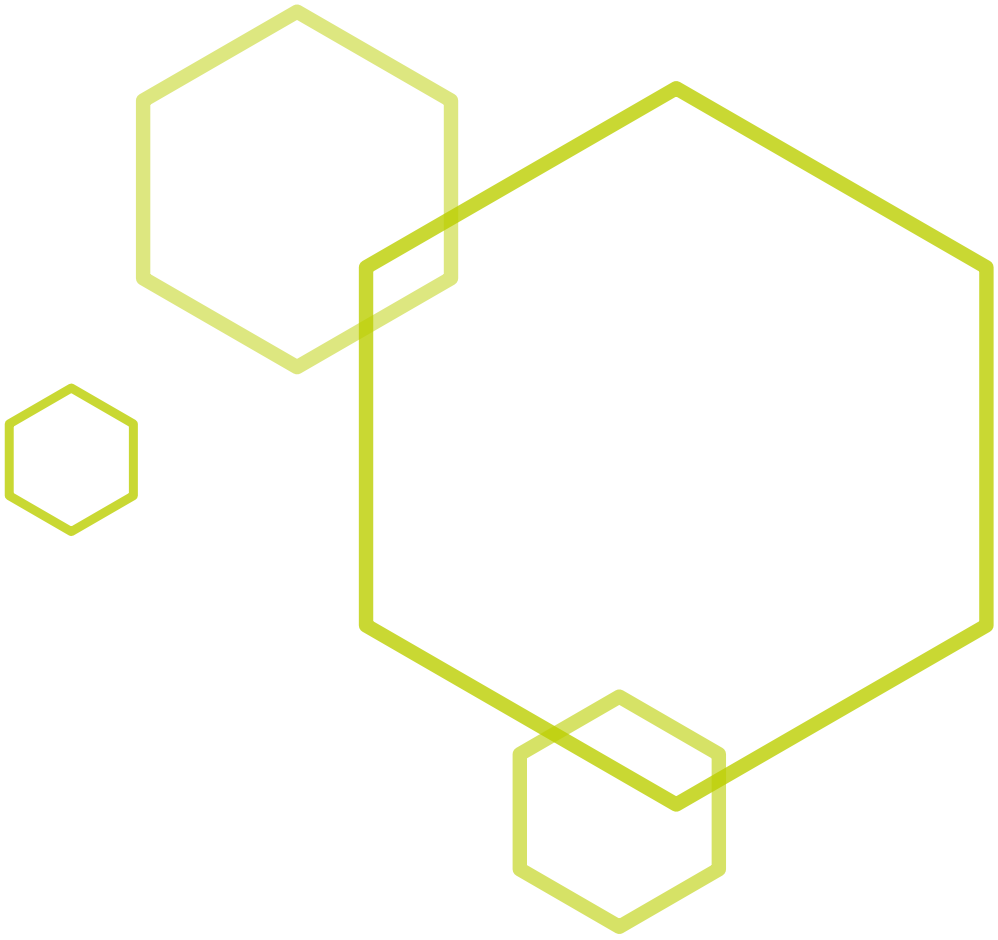
The role of diet in maintaining good health is widely acknowledged, but understanding the impact of individual components can be complex. The Western diet, which is high in fat and sugar but low in fiber, is a major contributor to chronic diseases in society. This unhealthy diet can disrupt the balance of the gut microbiota, causing dysbiosis, which in turn can harm the intestinal barrier and immune function. Certain dietary components, such as (poly)phenols, can have a prebiotic effect and shape the gut microbiota. These (poly)phenols can reach the colon intact and be broken down into bioactive microbial metabolites that promote health. Through their research on the prebiotic action of fruit (poly)phenols, the Industrial Partnership Chair supported by the Natural Science and Engineering Research Council of Canada and Diana Food (Symrise) has uncovered mechanisms by which cranberry and other small fruit (poly)phenols can improve intestinal barrier function and reduce the risk of metabolic endotoxemia and low-grade inflammation, which are linked to many chronic diseases.

Specifically, we have shown that a cranberry polyphenol extract ameliorates lipidemia, glycemia, and alleviate oxidative stress in mice fed an obesogenic diet and that this action derives from a favorable promotion of a beneficial gut microbiota. Interestingly, we have recently demonstrated, using a model of the human digestive system (SHIME), that a proanthocyanidin rich cranberry supplement, stimulates the production of beneficial mucosal colonic bacteria like *Akkermansia*, *Bifidobacteria*, *Roseburia*, and *Faecalibacterium*. These bacteria are now considered by many as favorable butyrate producing 2nd generation probiotics. This response results from a remarkable bacterial ecological shift leading to improved microbiota's function. For example, we demonstrate that a procyanidin rich extract directly alter the gut microbiota in such a way as to positively affect the gut bileacidome, thereby explaining its beneficial effect on cholesterol synthesis. We also show that cranberry procyanidins and procyanidin microbial metabolites (e.g. hydroxyphenyl g-valerolactones) are recognized topically by the host gut epithelium and induce the production of mucin, thereby favoring the growth of keystone bacteria like *A. muciniphila* (i.e., ecological niche engineering) and leading to a strengthening of the gut barrier. These results document the specific mode of action of procyanidins on metabolic health previously demonstrated in preclinical models and support the contention that cranberry they protect against cardiometabolic risk through a prebiotic action justifying their regular consumption.

Flash Oral Presentations



F 001 DEVELOPMENT OF A NOVEL (POLY)PHENOL-RICH DIET SCORE AND ITS ASSOCIATION WITH URINARY (POLY)PHENOL METABOLITES

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Background:

Few a priori dietary scores have been used to characterise (poly)phenol rich diets. This study aimed to develop a novel (poly)phenol-rich diet score (PPS) based on (poly)phenol intake from food frequency questionnaires (FFQs) and explore its relationship with circulating (poly)phenol metabolites.

Methods:

A total of 543 healthy free-living participants aged 18–80 years completed a FFQ (EPIC-Norfolk) and provided a 24 h urine. The PPS was developed based on the relative intake (quintiles) of 20 selected (poly)phenol-rich food items abundant in the UK diet, including tea, coffee, red wine, whole grains, chocolate and cocoa products, berries, apples and juice, pears, grapes, plums, citrus fruits and juice, potatoes and carrots, onions, peppers, garlic, green vegetables, pulses, soy and products, nuts, and olive oil. Foods included in the PPS were chosen based on (poly)phenol content, main sources of (poly)phenols, and consumption frequencies in the UK population. Associations between PPS and urinary phenolic metabolites were investigated using linear models adjusting energy intake, and multiple testing (FDR<0.05).

Result:

The total PPS ranged from 25 to 88, with a mean score of 54. Pearson correlations showed PPS is positively correlated with total urinary (poly)phenols metabolites (r=0.43 (95% confident interval (CI), 0.36, 0.50), p<0.001). There were 51 individual urinary metabolites significantly associated with PPS, including five flavonoids, three lignans, two other (poly)phenols, 39 phenolic acids, and two resveratrol metabolites. The total (poly)phenol intake also showed a positive association with PPS (stdBeta 0.32, 95% CI (0.24, 0.40), p<0.01). Significant positive associations were observed in 24 of 27 classes and subclasses of (poly)phenol intakes and PPS, with stdBeta ranging from theaflavins / thearubigins 0.12 (0.04, 0.20) to flavonols 0.43 (0.34, 0.51) (p<0.01).

Conclusion:

High adherence to the PPS diet is associated with (poly)phenol intake and urinary biomarkers, indicating the utility of the PPS to characterise diets rich in (poly)phenols at a population level.

Keywords:

(Poly)phenol-rich diet score; (Poly)phenol-rich food; Nutrients intake; Urinary metabolites

F 002 4-METHYLCATECHOL IS AN ACTIVE ANTIPLATELET DRUG IN FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

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Introduction:

Familial hypercholesterolemia (FH) is an inherited metabolic disease with elevated low-density cholesterol levels. High cholesterol levels are associated with hyperaggregability, and this further elevates cardiovascular risks. Current antiplatelet therapy has some limitations so research in this area is still needed. In this study we focused on confirmation of the effect of a flavonoid metabolite 4-methylcatechol (4-MC), previously shown to possess potent antiplatelet effect in healthy volunteers, in FH patients treated with different therapeutic modalities.

Material & Methods:

Blood from 15 patients suffering from FH treated with or without apheresis was used for comparison of the effect of 4-MC and acetylsalicylic acid (ASA) on platelet aggregation using impedance aggregometry.

Results:

4-MC was found to be on a molecular basis more effective than ASA on collagen and arachidonic acid induced platelet aggregation. The effect of 4-MC was improved by apheresis, moreover patients treated by apheresis had lower platelet activity than patients without apheresis treatment.

Conclusion:

4-MC is a possible candidate or template for a novel antiplatelet drug whose effect was already confirmed not only in a large healthy population sample but also in FH patients.

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F 003 UPSCALING APPLEWOOD EXTRACT PRODUCTION: ULTRASOUND ASSISTED EXTRACTION FROM LAB TO SEMI-INDUSTRIAL SCALE

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Introduction:

Annually, an apple grower must renew 5 to 10 % of his cultivation creating a waste stream in the form of apple wood. Apple wood is often considered a waste stream with low added value, yet it contains considerable amounts of bioactive components. The present contribution focusses on the extraction of polyphenols from applewood using an environmentally friendly technology, namely ultrasonic assisted extraction (UAE).

Objective and methods:

In a first phase of this study, solvent composition (ethanol 0–30–50–70 % v/v) and mass to volume ratio (3, 5 and 10) were optimized to obtain optimal extraction efficiency at lab scale (200 mL). Secondly, under the optimized conditions the UAE technique was scaled up to 1 L and finally 100 L. To this end, specific UAE probes are used, which deliver higher ultrasonic intensity, allowing to reduce extraction time and temperature while enhancing the extraction yields. For both goals, the generated extracts were evaluated in terms of total phenolic and total flavonoid content, as well as in terms of the antioxidant activity using *in vitro* assays. Moreover, for the scale-up experiment, characterization of the polyphenols was performed by HPLC-PDA.

Results:

First, the influence of solvent composition and mass to volume (m/v %) ratio on the extraction efficiency and extract composition were studied at lab scale (200 mL). Overall, a ratio of 3 and 5 m/v % resulted in higher amounts in polyphenol compounds compared to a ratio of 10 m/v %. Furthermore, higher levels in both polyphenols and antioxidant capacity were detected in the extracts produced in the presence of 30 v/v % ethanol mixture compared to pure water, a further increase in ethanol in the solvent mixture did not improve the extraction yield. Secondly, under the optimized conditions (30 v/v % ethanol-water; 3 and 5 m/v %), the UAE technique was scaled up to 1000 mL and finally 100 L. At 3 m/v %, the polyphenol yield reduced when scaled up to 100 litres, in contrast, at 5 m/v %, scaling up rather resulted in an increase. In order to identify and quantify individual phenolic compounds, HPLC-PDA analyses were performed. The dihydrochalcone phloridzin appears to be the major compound identified in all produced extracts. The level varies around 60 % relative to the total amount. Besides the chalcones, the main four flavonoids identified in the majority of the extracts include kaempferol-3-0-glucoside (20%), naringin (4–5%), (-) epicatechin (3–4%) and avicularin (7–8%). The relative proportions of the different polyphenols are hardly affected by the upscaling of the extraction process.

Conclusion:

This study can be considered unique as it applies a semi-industrial scale of UAE to generate valuable components from an agricultural waste stream. This is an important step in demonstrating the potential of UAE as an industrial process for the extraction of bioactive compounds.

F 004 GRAPE SEED PROANTHOCYANIDIN EXTRACT (GSPE) COULD IMPROVE METABOLIC SYNDROME SYMPTOMS IN LIVER BY MODULATING CIRCADIAN RHYTHMS OF ANTIOXIDANT-RELATED PARAMETERS IN A TIME-OF-DAY DEPENDENT MANNER

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Circadian rhythms are physiological, metabolic, and behavioral processes that follow a 24-hour cycle, as a response to the Earth's rotation movement, which determines the variations in light and temperature. These rhythms are orchestrated by the internal clock or central pacemaker found in the suprachiasmatic nucleus (SCN) of the hypothalamus, which synchronizes the peripheric clocks found in tissues such as liver, muscle, or adipose tissue. However, the circadian system can be altered due to feeding patterns like excess food, diet composition, or eating time. It has been suggested that these alterations may cause desynchronization between the central and peripheral clocks, leading to chronodisruption, which could contribute to the manifestation of typical metabolic syndrome alterations, including obesity or insulin resistance. Likewise, chronodisruption can also increase free radicals, hence the oxidative stress in tissues, which in the liver can contribute to cause non-alcoholic fatty liver disease (NAFLD). Polyphenols, such as proanthocyanidins, are secondary metabolites produced by plants under stress conditions. A Grape Seed Proanthocyanidin Extract (GSPE) has been described not only as a powerful antioxidant but also as an agent that may act against circadian dysregulation. Therefore, we wondered whether supplementation with GSPE could improve the oxidative stress status of diet-induced obesity rats due to the circadian modulation of oxidative stress-related parameters in the liver. For this purpose, ninety-six male 12-week-old Fischer 344 rats were housed under standard photoperiod and temperature conditions (12 h light and 23 ºC). During 9 weeks, 32 rats were fed a standard diet (STD) and 64 rats were fed a cafeteria diet (CAF). 4 weeks before euthanasia, rats received a daily dose of VH for the STD-fed rats or either VH or GSPE (25 mg/kg body weight) for CAF-fed rats at two different time points: when the light was turned on (ZT0) or when the light was turned off (ZT12). The animals were euthanized 1 h after light was turned on and every 6 hours (ZT1, ZT7, ZT13, and ZT19) to determine the diurnal profile. Interestingly, there were differences due to administration time. For example, at ZT0, the enzymatic activity of SOD and GPx1 showed circadian rhythmicity in all groups. Likewise, both *Sod1* and *Sod2* gene expression showed circadian rhythm in STD-fed rats. However, CAF disrupted the rhythmicity of both gene expression, which was partially ameliorated by GSPE restoring *Sod2* circadian rhythmicity. Furthermore, treatment with GSPE partially restored the circadian rhythmicity of GSH lost due to CAF. In addition, GSPE treatment at ZT12 decreased body weight gain in CAF-GSPE rats, as well as it restored the circadian rhythm of the SOD activity lost due to CAF. Therefore, these results suggested that GSPE treatment could also be effective for the treatment of NAFLD by reestablishing liver antioxidant circadian machinery in a time-dependent manner. Nevertheless further studies are still needed to set the molecular mechanisms associated to this interesting GSPE effect.

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F 005 INVESTIGATION OF ENDOGENOUS AND/OR EXOGENOUS PHENOLIC METABOLITES IN HUMANS USING (UN)TARGETED METABOLOMICS (ENDOPHENOL)

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(Poly)phenols contemplate a broad number of organic molecules with phenyl units, varying in structure complexity, that are widespread in beverages, fruit, and vegetables in the human diet. After ingestion, (poly)phenols follow the path of xenobiotics throughout the digestive system, and their route from mouth to bloodstream and urine results in their transformation – with an important participation of the gut microbiota – into a plethora of smaller catabolites (low-molecular weight phenolics, LMWP). LMWP appear in plasma in higher concentrations than their parent compound and are likely responsible for the reported biological activity of plant (poly)phenols. Meanwhile, some LMWP are also possibly produced by endogenous processes, like the vias for catecholamine production/degradation, the amino acid deportation system, and protein synthesis/breakdown, forming converging metabolites with those of plant (poly)phenols and contributing to the pool of LMWP in biological fluids. The current literature on (poly)phenol metabolism and bioavailability rarely includes discussion on LMWP origin (endogenous or exogenous).

This work proposes the characterization of LMWP in biological samples attempting to distinguish their origin based on the different urinary production of LMWP in the context of a controlled no-(poly)phenol diet, personalized in calories according to basal metabolic rates, and a single serving of a known source of (poly)phenols (primary outcome), considering parameters leading to interindividual variability (secondary outcomes), like the gut microbiota composition and genetic polymorphisms. Healthy adults between 20–40 years of age will be recruited for a randomized cross-over trial, each arm lasting 5 days, following the personalized-controlled diet for the whole period and consuming, on the third day, a dosage of coffee (source of (poly)phenols) or hot water (control). The primary outcome will be tackled by both targeted and untargeted LC-MS metabolomic analyses of urine samples to assess the pool of LMWP with and without a source of dietary (poly)phenols, and to check for potential (poly)phenolic metabolites not previously characterized, respectively, providing a comprehensive picture of the impact of coffee-(poly)phenol consumption on the urinary metabolome in humans. For the secondary outcomes, the foreseen analyses are i) fecal microbiota profiling and characterization to assess their influence over the endogenous and/or exogenous LMWP production and evaluate interindividual variability in metabolite production; ii) feces analysis for possible differences between produced but not absorbed exogenous (controlled-diet + coffee) and endogenous (controlled-diet + water) LMWP; and iii) genotyping for detecting variations that could be associated with phenotype or response to the intervention, specifically for (poly)phenol metabolism. In general, epidemiological studies have attributed many biological effects to (poly)phenol intake on noncommunicable chronic disease prevention, and efforts have been made to understand their role in the health outcomes associated with a plant-rich diet. Accordingly, steps must be taken to understand and consistently report circulating LMWP species, concentrations, and possible sources, to further comprehend their role in nutrition and health, also advancing the field of personalized nutrition.

This work is part of the PNRR Partnership Extended ON FOODS consortium (ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods).

F 006 EXPLORING THE POTENTIAL OF HYDROXYTYROSOL AS AN ADJUVANT AGENT IN COLORECTAL CANCER TREATMENT: EFFECTS OF ITS COMBINATION WITH CHEMOTHERAPY DRUGS ON LOVO SPHEROIDS

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Colorectal cancer (CRC) represents the second most lethal cancer worldwide, accounting for nearly 1 million deaths per year. Despite current advances in diagnosis and treatment, resistance to chemotherapy and associated adverse effects remains one of the biggest challenges in the management of patients with metastatic CRC. To overcome these limitations, scientists have been exploring other treatment strategies involving the combination of conventional anticancer drugs with natural bioactive compounds, such as polyphenols. Hydroxytyrosol (HT), one of the main phenolic compounds of virgin olive oil, has been reported to induce promising antioxidant and antitumoral effects on CRC cells by promoting apoptosis and inhibiting cell proliferation. Nevertheless, there are almost no studies evaluating the therapeutic potential of HT as an adjuvant agent in chemotherapy. Therefore, this work aimed to explore the anticancer effects of HT alone and in combination with conventional chemotherapeutic drugs (5-Fluorouracil, Irinotecan and Oxaliplatin) using 2D cultures (monolayers) and 3D models (spheroids) of a CRC human metastatic cell line (LoVo). The antiproliferative activity and cytotoxic effects (on confluent Caco-2 cells) were assessed using cell viability assays (PrestoBlue). Spheroids were generated by a stirred culture system (spinner flasks) and were morphological and phenotypically characterized using fluorescence and phase-contrast microscopy as well as cell viability/counting methods (trypan blue and crystal violet staining).

Our data indicated that LoVo spheroids (of approximately 200 µm in diameter) were highly compact and exhibited high viability (≥85%) throughout the 8 days of culture. Also, the immunostaining showed that most cells in the spheroids expressed epithelial markers, such as F-actin and E-cadherin, while only a subset of cells presented stemness (CD133) and mesenchymal (vimentin) characteristics. Complementarily, hypoxia regions and an increased number of apoptotic cells (positive for caspase-3) were detected in the core area of the spheroids. We are currently characterizing these models concerning the expression of genes related to stemness (NANOG, OCT4), Epithelial-Mesenchymal Transition (VIM, SHH, TGFβ1), Sonic Hedgehog Pathway (GLI1, PTCH1), proliferation (CCNA2) and other key biomarkers of metastatic CRC by qRT-PCR analysis.

Regarding the antiproliferative activity studies, results showed that HT inhibited LoVo cells' proliferation in a dose-dependent manner after 72 h of incubation, revealing half maximal effective concentration (EC_{50}) values of 103 µM in monolayer cultures and 1454 µM in spheroids. The chemotherapeutic drugs also induced growth-inhibitory effects on both cell models, with Oxaliplatin showing a significantly more pronounced effect (EC_{50}: 9.2 µM) on LoVo spheroids than Irinotecan (EC_{50}: 784 µM) and 5-Fluorouracil (EC_{50}: 917 µM). Additionally, combination experiments showed that HT was able to potentiate the antiproliferative activity of all the drugs on LoVo spheroids when administered at concentrations above 500 µM. Further studies will be conducted to evaluate the impact of HT and these combinations on targeting cancer stemness and reducing cell migration and invasion.

Overall, this research provides new relevant insights into the anticancer activity of HT and its potential beneficial effects in the prevention and treatment of metastatic CRC.

F 007 LIGNAN-DERIVED METABOTYPES AND THEIR ASSOCIATION WITH THE CARDIOMETABOLIC HEALTH STATUS

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Background:

A growing body of evidence underlines the potential beneficial effects of various (poly)phenol-rich foods against chronic diseases, including cardiovascular diseases, type II diabetes, neurodegeneration, and some cancer types. Nevertheless, conclusions regarding their effectiveness remain unsolved due to several limitations, such as the different metabolic profiles in response to the consumption of these food bioactives. Individuals showing the same metabolic pathway can be clustered into phenolic metabolic phenotypes (the so-called metabotypes), while different metabotypes may be associated with different disease risk levels.

Objectives:

This study focuses on the identification of lignan-derived metabotypes and how the existence of different metabotypes may be related to the cardiometabolic health status.

Methods:

Three hundred volunteers (18–74 y.o., BMI 18–35 kg/m^2) without cardiometabolic diseases or gastro-intestinal surgeries were enrolled in an intervention study. They were subjected to an oral (poly)phenol challenge test (OPCT), consisting in an acute supplementation of several classes of dietary (poly)phenols in the form of 3 tablets. Dietary and lifestyle information, anthropometric and body composition measurements, clinical data, and biological samples such as blood, urine, and faeces, were collected. Cardiometabolic health biomarkers and whole-genome genotyping data were achieved by analysing blood samples. Urine samples were analysed through UPLC-IMS-HRMS to assess the individual urinary excretion of phenolic metabolites, allowing clustering according to lignan-derived metabolites and the whole pool of phenolic metabolites. Moreover, the microbial profiling of faeces samples was carried out to define the microbiota composition at species level. Cardiometabolic risk scores were also computed.

Results:

Up to 298 volunteers finished the study: 56.7% were women and the average (± SD) age was 40.6 ± 16.2 years. The study population presented BMI values close to those of the Italian population, which may increase the external validity of the study: 72.7% were normal weight, 22.5% overweight and 4.8% obese. Phenotyping according to the whole set of phenolic metabolites accounted for the role of some lignan metabolites as proxy of aggregated phenolic metabotypes. Preliminary results on lignan-derived metabotypes pointed out to high and low enterolactone-3-glucuronide producers, while other enterolactone and enterodiol derivatives showed different patterns. The cardiometabolic risk of individuals falling within different metabotypes was estimated. The associations between the aforementioned metabotypes and health determinants such as age, sex, BMI, smoking habits, biochemical markers of the lipid and glucose panel are being explored, in order to broaden our knowledge on the role of interindividual variability in lignan metabotype formation.

Conclusions:

These preliminary results showed that enterolactone-3-glucuronide is the main discriminant when clustering individuals on the basis of lignan-derived metabotypes. Further analyses are underway to discover other potential metabolites with this role and to better understand the complexity of the lignan metabolic pathway among individuals.

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F 008 BARLEY-BASED DIETS RICH IN BIOACTIVE COMPOUNDS ENHANCE GUT HEALTH THROUGH MODULATION OF MICROBIOTA AND INFLAMMATION

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Background:

Barley has gained recent interest in the market niche of healthy food products due to the functional properties of its bioactive compounds such as β-glucans and polyphenols. Novel purple genotypes of barley biofortified in anthocyanins (ACN) have been developed that could have potential beneficial effects on gut health.

Objectives:

The main objective of this study is to explore the effect of diet supplementation of purple barley as a whole grain cereal or its isolated fractions (bran and endosperm) on promoting healthy gut microbiota and inflammation in a murine model.

Methods:

50 male and female Balb/c (BALB/cAnNCrl) mice were assigned into 5 experimental groups (10 mice/group, 5 males and 5 females). During 6 weeks, mice were fed according the following diets: a standard purified diet (SD), supplemented with white rice (IRC), supplemented with whole grain barley (WBG), supplemented with the outer fraction of the grain (bran) rich in ACN (BB), and supplemented with the inner fraction or endosperm (pearled grain) rich in β-glucans (PG). Faecal samples were collected at the beginning of the study, at week 3, and at the end of the study (week 6). At the latter time, ileum content samples were also collected. DNA was extracted from faecal (3 time-points) and iliac (1 time-point) samples. The V3-V4 hypervariable region of the 16S rRNA gene was sequenced using an Illumina MiSeq Sequencer. Raw sequences were processed, and amplicon sequence variants were identified using the DADA2 pipeline. Further, the microbial diversity was assessed at the genus level using the phyloseq and ANCOMBC packages in R. Serum lipopolysaccharide-binding protein (LBP) and C-reative protein (CRP) were analyzed using ELISA kits and cytokines interleukin IL-4, IL6, TNF-alfa and IFN-gamma were analyzed using milliplex.

Results:

Microbial alfa-diversity in faeces was significantly higher than ileon. Significant differences were detected in β-diversity between diets ($p<0.001$). Genera related to butyrate production and protection against inflammatory diseases, such as *Akkermansia, Romboutsia,* and *Mucispinillium,* were differentially abundant in mice supplemented by barley compared to controls. Significant differences were detected in the overall microbiota composition of fecal samples between time points. Alfa-diversity increased with time.

Regarding plasmatic LBP, only the PG group presented significantly lower values compared to control groups. The inflammatory marker TNF-alfa was significantly reduced in BB compared to SD group in both males and females, and IL-6, IL-4 and IFN-gamma were also significantly reduced after BB compared to control groups only in females.

Conclusion:

Barley diet supplementation had a positive impact in mice gut microbiota diversity and composition, especially when diet was supplemented with whole grain or pearled grain, both rich β-glucans. Moreover, isolated bran of purple barley rich in ACN intake had a positive impact on systemic inflammation. Purple barley may reduce the inflammation associated with gut microbiota modulation; thus, this new genotype offers interesting avenues to meet the demand for healthier cereal products.

F 009 SPECIFIER PROTEIN AND MYROSINASE ACTIVITY AND GLUCOSINOLATE PROFILE DETERMINE THE OUTCOME OF GLUCOSINOLATE HYDROLYSIS PRODUCT FORMATION AND PROFILE IN KOHLRABI TISSUES

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Brassica vegetables such as kohlrabi, cabbage and broccoli are widely consumed and have numerous health beneficial effects, linked to bioactive compounds formed upon glucosinolate (GLS) hydrolysis. GLS are sulfur-rich thioglucosides that undergo myrosinase-catalyzed hydrolysis upon tissue damage by herbivores or pathogens. Isothiocyanates (ITCs) are often the main GLS breakdown products. However, the formation of other biologically active breakdown products like simple nitriles and epithionitriles occurs in the presence of specifier proteins such as nitrile-specifier protein (NSP) and epithiospecifier protein (ESP). While ITCs exhibit anticarcinogenic, anti-inflammatory, and antimicrobial properties, simple nitriles and epithionitriles have less health beneficial potential. Therefore, increasing the proportion of ITCs in *Brassica* vegetables is preferable. However, the tissue-specific impact of specifier proteins on the GLS degradation outcome is still unclear.

To enhance our understanding of specifier protein function, we used an LC-MS-based approach to investigate the protein abundance patterns of *BoNSP1* and *BoNSP2*, *BoESP1-3*, *BoESM1-like* protein and myrosinase in nine mature kohlrabi organs (leaf midvein, leaf lamina, leaf margin, leaf stalk, bulb core, bulb middle part, bulb peel, stem and root). Further, we extracted GLS and GLS hydrolysis products from the lyophilized plant material and analyzed them by UHPLC-DAD and GC-MS, respectively. We correlated the protein abundance patterns with the GLS hydrolysis product formation. Also, the ESP and myrosinase activity in all nine kohlrabi organs were determined.

The tissue-specific GLS profiles were consistent with the GLS hydrolysis product profiles. The specifier protein and myrosinase activity fit the GLS hydrolysis product formation. We observed differing ESP activities across all organs, with the highest activity in the bulb core and barely detectable activity in the stem. The myrosinase activity was consistent with the abundance in all organs except the stem. Nitrile formation was predominant in five of the nine organs analyzed (leaf midvein, leaf lamina, leaf margin, bulb core and bulb middle part). ITCs were the main breakdown products formed in the other four kohlrabi organs (leaf stalk, bulb peel, stem and roots).

In conclusion, this study demonstrates that the specifier protein and myrosinase activity and GLS profile all play a crucial role in determining the resulting GLS hydrolysis product profile. With a better understanding of how specifier proteins function, we may be better equipped to optimize the GLS degradation pathway and produce *Brassica* vegetables with an increased ITC content and potentially more health beneficial effects.

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F 010 PHYTOCHEMICAL CHARACTERIZATION AND VOLATILE COMPOSITION OF 38 SAFFRON (*Crocus sativus* L.) STIGMAS PRODUCED IN ALGERIA: IDENTIFICATION OF COMPOUNDS WITH ANTIOXIDANT EFFECT

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Saffron is a well-known spice obtained from the dried stigmas of *Crocus sativus*. Due to the labor-intensive practices required for its production, it is also considered one of the world's most expensive spices ("the red gold" spice). Previous studies already demonstrated that the stigmas are an important source of unique bioactive compounds, including major components like crocins, picrocrocin (and safranal, as a derivative), and crocetin, with a wide range of therapeutic properties: anti-inflammatory, antioxidant, cardioprotective, immunoregulatory, antidepressant, anxiolytic, and neuroprotective. The concentration of these compounds may vary according with the geographic origin of the samples, the post-harvest treatment or storage conditions and thus the health promoting effect of the stigmas could be different.

This work was developed within the PRIMA SAFFROMFOOD project and aims to compare the phytochemical and volatile composition of 38 Algerian saffron stigmas with different origins and post-harvest treatments using high-throughput chromatographic techniques, namely UHPLC-DAD-UV/ED and GC-MS/MS, respectively. The antioxidant capacity was also evaluated in all samples using two different chemical assays (Oxygen Radical Absorbance Capacity (ORAC) and hydroxyl radical scavenging capacity (HOSC)) and a cell-based assay (cellular antioxidant activity (CAA) using confluent Caco-2 cells).

Results obtained by UHPLC-DAD showed that the total crocin content (at 440 nm) varied from 46.9 ± 0.42 $mg_{crocin\ 1\ equiv.}/g_{dw}$ and 239.9 ± 6.5 $mg_{crocin\ 1\ equiv.}/g_{dw.}$ Compounds identified include *trans* and *cis*-crocetin, picrocrocin, HTCC (2,6,6-trimethyl-4-hydroxy-1-carboxaldehyde-1-cyclohexene) and kaempferol-3-sophoroside-7-glucoside that were quantified in all samples. Total phenolic composition was also determined (Folin-Ciocalteu assay) varying from 17.7 ± 0.64 $mg_{GA\ equiv.}/g_{dw}$ and 31.5 ± 1.62 $mg_{GA\ equiv.}/g_{dw}$. The volatile fraction of stigmas was predominantly composed by safranal and alpha-isophorone, accounting for about 34% and 10% of the total volatile compounds, respectively. In these stigma samples, up to 100 volatile compounds were detected by GC-MS/MS. Concerning the antioxidant activity, the ORAC value of stigmas varied from 344 ± 47 µmol TEAC/g_{dw} and 601 ± 79 µmol TEAC/g_{dw}, while HOSC value varied from 350 ± 43 µmol TEAC/g_{dw} and 593 ± 70 µmol TEAC/g_{dw}. Among all compounds only crocin 1 (*trans*-4-GG), crocin 2 (*trans*-3-Gg) and *trans*-2-G were detected by UHPLC with electrochemical detection, suggesting that these crocins may have an important contribution to the antioxidant potential of samples. Multivariate Data Analysis tools were used to putatively identify compounds that may be responsible for the antioxidant capacity of saffron samples.

The data generated herein will provide relevant knowledge to further understand the health promoting effect of saffron stigmas and to design green extraction processes to develop high added value ingredients from this medicinal plant.

F 011 PROTEIN-PHENOL INTERACTION AS A STRATEGY TO ELIMINATE THE IMMUNOGENIC GLUTEN PEPTIDES

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Increasing numbers of patients with Celiac disease and non-celiac gluten sensitivity have led to an increase in the consumption of gluten-free and low-gluten products recently. Due to the high proline content of gluten, which makes it resistant to digestive enzymes, partial digestion of gluten results in the production of immunogenic gluten peptides. Proline residues are prone to react with phenol compounds in terms of protein-phenol interaction, on the other hand. Protein-phenol interactions might take place between proteins and phenol compounds through covalent and non-covalent bonds. Under alkaline conditions, covalent interactions are stimulated through the addition of electrophilic quinones, which are produced during the oxidation of phenolic compounds, to the side chains of proteins such as amino and thiol compounds. This study aims to stimulate the gluten-phenol interaction and investigate the possible effects on immunogenic gluten peptides.

For this purpose, gluten was mixed with green tea extract (GTE) phenol compounds at 50 °C for up to 3 hours. The incorporation of GTE phenol compounds to the gluten was confirmed by monitoring the changes in the total antioxidant capacity (TAC), in the amount of amino and thiol groups, and in the thermal properties of the gluten.

The TAC of GTE-treated gluten was much higher than that of untreated gluten, whereas the amount of free thiol and amino groups in untreated gluten decreased. The increased thermal stability of gluten treated with GTE was further validated by differential scanning calorimetry (DSC) analysis. These findings indicated that the gluten-phenol interaction was successful under these conditions. Gluten samples treated with GTE were *in-vitro* digested to better understand the potential consequences of the gluten-phenol interaction on the immunogenic peptides of gluten. The amount of the most immunogenic gluten peptide (33-mer) was significantly reduced (57%) after 2 hours of treatment of gluten with GTE at pH 9. These results suggested that protein-phenol interaction is a promising method for reducing the immunogenicity of gluten and can be applied for the development of gluten-free or low-gluten food products for those who have Celiac disease or non-Celiac gluten sensitivity.

F 012 ROLE OF GUT MICROBIOTA IN THE ANTIHYPERTENSIVE EFFECT OF PROTEIN HYDROLYSATES IN SHR

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In recent decades, many studies have aimed to understand the role of the gut microbiota in the development of various non-communicable diseases, including hypertension. However, their relationship and the underlying mechanisms remain unknown. Hypertension affects a significant percentage of the global population and is considered a major risk factor for cardiovascular disease. Although there are efficient antihypertensive drugs, some of them can produce several side effects and are not recommended for prehypertensive patients. Therefore, the identification of new preventive and treatment strategies is of interest, particularly if they are derived from natural sources. In this context, bioactive peptides obtained from food or agro-food industry by-products have emerged as interesting alternatives for managing hypertension. In our previous study, several protein hydrolysates obtained from three by-products of the agro-food industry showed angiotensin-converting enzyme inhibitory activity and were able to modulate fecal microbial composition *in vitro*. Thus, the objective of this study was to investigate the *in vivo* antihypertensive capacity of protein hydrolysates obtained from agro-food by-products and evaluate the influence of the gut microbiota on their activity. The antihypertensive activity of 5 hydrolysates (Three of them obtained from two different animal by-products and two obtained from a vegetable by-product) at an acute oral dose of 55 mg/kg was determined in spontaneously hypertensive rats (SHR) using a telemetry system. Tap water and captopril (antihypertensive drug, 55 mg/kg) were used as the controls. After one-week of washout period, animals drank a broad-spectrum antibiotic cocktail daily for 1 week to eliminate the gut microbiota, and the aforementioned experimental design was repeated. Two of the hydrolysates exhibited antihypertensive activities. The hydrolysate from the meat by-product mainly reduced blood pressure during the first 8 h post-administration. However, the hydrolysate obtained from the plant-based by-product showed its antihypertensive effects after at this 8 h post-administration. Interestingly, when the gut microbiota of the animals was severely reduced by antibiotics, the blood-pressure lowering effect of both hydrolysates disappeared, showing a similar pattern in blood pressure reduction to that observed in the water group. No significant differences were observed between the two water groups (with and without microbiota). These findings show that gut microbiota seems to be involved in the antihypertensive activity of these bioactive peptides.

F 013 A COMPARATIVE STUDY OF EFFECTS OF INULIN AND CHICORY ON GUT HEALTH IN PIGLETS DURING THE WEANING PERIOD

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Inulin is well known for its positive impact on porcine gut health by promoting the growth of beneficial bacteria that potentially reduce inflammation by producing short-chain fatty acids (SCFAs) such as butyrate. Thus, inulin is a promising prebiotic commonly extracted from chicory root but associated with high extraction costs. Therefore, the objective of this study was to compare the effect of crude chicory flour to inulin on gut health during weaning, a critical period in the life of pigs, causing low-grade inflammation. Moreover, chicory flour contains several bioactive molecules like polyphenols, sesquiterpene lactones, essential oils, etc., with potential health benefits.

Two *in vivo* experiments (E1 and E2) were performed, each consisting of 72 castrated male piglets, weaned at day 21 and subsequently divided into three groups with *ad libitum* feed: control (Ctrl), inulin (IN) and chicory flour (CHI). For IN and CHI, a daily supplementation was done by oral force-feeding with the 'inulin content' increasing weekly (W) (W1: 0.5 g/day, W2: 1 g/day, W3: 1.5 g/day) (E1) or double this dose (E2). The 'inulin content' was equated for CHI and IN groups. At the end of W1 and W3, 8 piglets per treatment were euthanized to collect ileum and colon tissues for gene expression analyses and content for SCFA & microbiota 16s sequencing.

In E1, the CHI group showed lower average daily calorie intake (kcal/day) only on W3, while in E2, it was lower than Ctrl and IN for all three weeks. Interestingly, this did not cause any significant difference in weight gain on W2 and W3. In E2, the severe diarrhoea occurrence was significantly lower for CHI compared to IN and Ctrl in W3. The villi-to-crypt ratios indicated that higher doses of CHI had a beneficial effect. The total SCFAs were significantly higher in IN than CHI and Ctrl in W1 in the colon. However, both groups showed significantly higher butyrate production in W1 and W2 compared to Ctrl in E2.

IN and CHI elevated beneficial microbiota throughout both experiments. In E1, the IN and CHI significantly increased health-promoting genera in W1 (*Fibrobacter*, *Prevotella*, *Acidaminococcus*) and W3 (*Gastranaerophilales*, *Dialister*, *Megasphaera*). Interestingly, in E2, CHI had a more dominant effect on increasing the abundance of health-promoting genera like *Catenisphaera*, *Butyricicoccus*, and *Ruminococcaceae_UCG-008* compared to IN. In E2, CHI also significantly decreased harmful genera like *Erysipelotrichaceae_UCG-002* and *Slackia*.

For E1 and E2, 48 genes (proinflammatory, inflammation signalling and barrier integrity genes) in the ileum were analysed by high-throughput qPCR. In E2, on W3 several pro-inflammatory genes were downregulated in IN and CHI (CXCL10, IL18, TNFα). For inflammation signalling genes, IN and CHI downregulated MyD88 and NF-κB1 on W3, thus inhibiting the inflammatory signalling pathway, leading to a decrease in the production of pro-inflammatory cytokines. Despite equating the inulin content in both groups, CHI supplementation had similar or better effects than IN and was a helpful supplement in alleviating weaning stress. Therefore, chicory might be a promising cost-effective alternative to inulin to improve gut health in weaned piglets.

F 015 IMPACT OF REDUCED-PHOSPHATE-AVAILABILITY ON ESSENTIAL MICRONUTRIENTS IN MAIZE FOR HUMAN CONSUMPTION

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Phosphorus (P) is an essential plant macronutrient. P deficiency can suppress or delay plant growth and maturity, leading to low yields and smaller fruits. Due to its importance in agriculture, P is a key element in fertilizers. Nonetheless, the effect of P deficiency and fertilization on the contents of nutrients important for human nutrition in many crops is not known. We therefore investigated if P fertilization of maize grown on P sufficient soils alters the contents of fatty acids, vitamin E and carotenoids in the grains of the hybrids Es Metronom, LG30258 and Ricardinio. Additionally, we investigated if the P fertilization influences the lipid oxidation of whole maize flour produced from the same hybrids grown under the same conditions, performing an accelerated shelf life test. In LG30258 grains, the most P-sensitive of the tested hybrids, grown without P fertilizer, α-linolenic acid decreased by 24%. P fertilization did not influence the concentration of vitamin E, carotenoids and all other fatty acids in any of the tested hybrids. Additionally, the P fertilization had only a minor impact on lipid oxidation. Here we show that the exclusion of an external P source has little impact on the nutritional composition of maize grains grown on P-sufficient soils. However, the effect of P deficiency is not yet known and warrants further investigation.

F 016 EFFECT OF PROCESSING METHOD ON HEAVY METAL CONTENT IN SPECIALTY COFFEE

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Coffee beverages are important to human health due to their antioxidant properties. Basic processing methods of specialty coffee have recently been supplemented by new innovative processing methods, e.g., anaerobic fermentation and carbonic maceration. Anaerobic fermentation and carbonic maceration rapidly change the coffee fruit, resulting in a flavor much different than traditional fermentation methods. Heavy metals are evaluated in coffees because coffee plants can absorb and store them in the roots, shoots, or grains. However, the bioavailability and occurrence of heavy metals are influenced by other factors such as soil pH, organic matter, soil texture, and interactions among elements. In contrast to roasted coffee, limited studies have compared the contents of heavy metals in green beans because some metals in green beans change after roasting depending on the degree of roasting. Heavy metals are elements that have densities >5 g/L and atomic weights between 63.5 and 200.6 g/mol. They are extremely harmful to human health, even in very low concentrations because they accumulate in the food chain. Some metals, including copper (Cu), chromium (Cr), cobalt, nickel (Ni), and zinc, are biologically essential to organisms at low concentrations. The contents of heavy metals in green and roasted coffees processed using the new methods have rarely been reported. We thus hypothesized that the origin of coffee and the methods of roasting and processing could strongly affect the contents of heavy metals in the beans. The aim of this study was to determine the effect of the processing method (natural, washed, honey, anaerobic fermentation, carbonic maceration) on the content of heavy metals in specialty coffee beans from different countries of origin. Heavy metals were identified using the multi-element technique of inductively coupled plasma mass spectrometry. Coffee roasting had a significant effect ($p < 0.001$) on the content of Hg, Al, Ni, Cd, and Pb. The content of Al was high in coffees from Kenya, Rwanda, and Guatemala, and Ni content from Rwanda in roasted coffees processed by anaerobic fermentation. The highest Cd content was in Guatemalan coffee processed by carbonic maceration. The highest Pb values were found in roasted Ethiopian coffees processed washed, natural, and anaerobic fermentation methods. The geographical origin of coffee had a significant effect ($p < 0.001$) on the content of Hg, Al, Ni, Cd, Pb, and Cr. Still, the values of other heavy metals in coffee remain within the recommended limits. The importance of monitoring heavy metals should be part of the entry controls for green coffees entering the market, even though further processing of the coffee, such as roasting, significantly reduces their amounts. The method of specialty coffee production can preserve the content of bioactive compounds but also minimize harmful contaminants. Drinking high-quality and healthy coffee should be a goal for the future. This study was supported by funds from The Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0073/22).

F 017 NUTRITIONAL, FUNCTIONAL AND SAFETY COMPARISON OF AN AUSTRALIAN NATIVE GRAIN WITH WHOLE WHEAT

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Background:

The Australian native foods industry is currently experiencing unprecedented growth. This is largely due to increasing consumer interest in foods that are naturally grown, highly nutritious and contain beneficial functional properties, such as high antioxidant content. As mainstream awareness grows, the functional and medicinal properties attributed to Australian native plants used as foods and/or that feature in various traditional pharmacopoeias utilised by Aboriginal Australia, are now being sought after. However, the properties attributed to many of these plant foods are largely undocumented in written format and only limited research has been done to verify their nutritional and functional properties or their dietary safety. This study is part of an ongoing research project that aims to systematically analyse the nutritional and functional properties and dietary safety of a range of native Australian plant foods that are used traditionally by various Aboriginal groups and are today being considered for commercial markets.

Objectives:

In this study, the nutritional, functional and safety profile of a native grain variety that is used traditionally by various Aboriginal groups of Australia has been directly compared to a commonly consumed comparator of whole wheat (*Triticum aestivum*).

Methods:

Ground whole wheat and native grains were sequentially processed with increasingly polar solvents using sonication-assisted solvent extraction to produce five extracts in *n*-hexane, ethyl acetate, acetone, methanol and water. Chemical compositional analysis of these extracts was performed for the identification and quantification of chemical classes and individual analytes contained within the edible plant materials, including liquid chromatography (LC) coupled to mass spectrometry (MS) and tandem MS. A range of *in vitro* screening assessments using human cell lines were also performed on these extracts to provide an indication of potential bioactivities, including antioxidant potential, reactive oxygen species generation, cytokine release profiles and cytotoxicity. Complete nutritional profiles were elucidated for whole grains, including analysis of proximates, macro- and micro-minerals, vitamins, total polyphenols and gluten content.

Results:

Our findings showed that the native grain variety is nutritionally superior to commonly consumed whole wheat, containing double the amount of protein and total fats, and higher levels of macro- and micro-minerals. Importantly, the native grain was found to be gluten-free, while also possessing 2.5-fold greater total polyphenol content. The native grain exhibited greater antioxidant potential in the *in vitro* test system, while other bioactivities were generally similar. Our safety assessment utilising substantial equivalence has shown that the native grain did not elicit toxic effects above that seen for the commonly consumed wheat comparator.

Conclusion:

This study suggests that the characterised native grain species is a viable crop for further commercial development. Our findings that this grain is both gluten-free and nutrient-dense suggest that it could be utilised as a value-adding product to improve the nutritional and bioactive contents of gluten-free grain products. This project is also the first to comprehensively address the safety data gap for Australian native foods, which is a prohibitive hurdle in successfully getting such products to market, and in doing so has provided supporting evidence that this native grain variety is safe for general consumption.

F 018 ANTI-INFLAMMATORY EFFECT OF POLAR AND NON-POLAR EXTRACTS OF AFRICAN GREEN LEAFY VEGETABLES ON LPS-STIMULATED THP-1 MACROPHAGES

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Stunting in children is associated with low-grade intestinal inflammation. This form of chronic malnutrition is associated with malabsorption of nutrients and with altered immune function. A diet rich in antioxidant and anti-inflammatory compounds may have the potential to prevent inflammation. We selected two African green leafy vegetables (GLV) (cassava and roselle leaves), previously identified as having interesting antioxidant capacity, to evaluate their anti-inflammatory properties.

Polar (ethanol/water, 80/20) and non-polar (ethanol/hexane, 60/40) extracts were obtained by solid-liquid extraction of freeze-dried GLV. Total polyphenol content (TPC) were obtained using Folin-Ciocalteu procedure and phenolic compounds were identified by UHPLC-DAD-MS^2. Carotenoid content was characterized by HPLC. Anti-inflammatory activity of extracts (6.25–50 µg dry matter/ml) was determined using a cell culture model of human macrophages (monocyte cell line THP-1). After differentiation of monocytes into macrophages (48 h) with PMA (20 ng/mL), inflammation was triggered by exposure to LPS (100 ng/mL) for 4 or 18 h. The production of pro- (TNF-α, IL-6, IL-8) and anti-inflammatory (IL-10) cytokines was measured by ELISA. The lactate dehydrogenase (LDH) level was measured in the medium as marker of cytotoxicity.

Cassava and roselle leaves had high levels of TPC (2500 and 2720 mg GAE/100 g DM, respectively). Roselle leaves contained the greatest diversity of phenolic compounds belonging to two classes of phenolic compounds: the flavonols (60%) and the hydroxycinnamic acids (40%). Cassava leaves contained mainly flavonols (83%), flavones (9%) and only 8% of hydroxycinnamic acids. Cassava leaves had the highest lutein content (48 mg/100 g DM) while there were no difference in β-carotene content (32–35 mg/100 g DM) between the two GLV. LDH activity demonstrated the absence of cytotoxicity whatever the concentration of extracts (<5 mU/ml). This model showed a concentration-dependent inhibition of TNF-α and IL-6 production by both polar and non-polar extracts of cassava and roselle leaves. Polar extract of cassava and roselle at 50 µg/ml inhibited, respectively, 34 and 21% of TNF-α production and 56 and 51% of IL-6 production compared to the positive control. Inversely, non-polar extract of cassava inhibited TNF-α production less strongly than roselle extract (22 and 35%, respectively) while they had the same strong effect on IL-6 production (65% of inhibition). Moreover, non-polar extracts showed a stronger inhibition of IL-8 production (40 and 33% for cassava and roselle) than polar extracts (cassava, 27%) while roselle extract had no effect. However, GLV extracts had no effect on IL-10 production. This lower production of inflammatory cytokines suggests a general inhibitory effect on macrophages activation which could be due to polyphenols and carotenoids.

Under these experimental conditions, non-polar extracts exhibited greater anti-inflammatory activity than polar extracts. Polar extract of cassava leaves had a stronger anti-inflammatory effect than those of roselle, may be due to their high content of polyphenols, especially flavonoids. Concerning non-polar extracts, no clear difference appeared between the two GLV. According to these results, regular consumption of GLV in sub-Saharan Africa may contribute to reduce inflammatory markers. Further investigation is in progress using an *in vitro* tri-culture model of gut inflammation to confirm these anti-inflammatory effects.

F 019 INVESTIGATING THE BIOACTIVE PROPERTIES OF APPLE POMACE: UNLOCKING ITS POTENTIAL

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Apple (*Malus* spp.) stands as one of the most globally esteemed fruits, with a significant portion of its production being processed and converted into juice or cider. The process of extracting apple juice engenders a solid residue known as apple pomace, representing around 30% of the original fruits (1). Consequently, this industrial activity generates large quantities of underused bio-residues, which harbor remarkable potential owing to the presence of bioactive compounds. Bearing that in mind, this work aims to screen the apple pomace (AP) as well as the individual apples (IA) focused on finding out ways to use them at their full potential. The nutritional profile (protein, ash, fat, dietary fiber, carbohydrates, and energy content) was determined using official methodologies (AOAC). The chemical profile encompassed soluble sugars (HPLC-RI), fatty acids (GC-FID), tocopherols (HPLC – fluorescence), organic acids (UFLC-PDA), and phenolic compounds (HPLC-ESI-Orbitrap-MS). The antioxidant capacity of the extracts was evaluated by two *in vitro* assays: the ability to inhibit the formation of thiobarbituric acid reactive substances (TBARS) in brain cell homogenates; and oxidative hemolysis inhibition (OxHLIA). The nutritional evaluation showed that AP and IA are rich in water, with a moisture content of around 78% and 83%, respectively followed by dietary fibers with values on average 7.1 g/100 g fw (fresh weight) for AP and 2.5 g/100 g fw for IA. Values close to 0.55 and 0.25 g/100 g fw were obtained for proteins of AP and IA. Ash showed minimal variations, representing about 0.4 g/100 g fw and the energy value was around 70 kcal/100 g fw for all samples. The sugar profile was composed of fructose, sucrose, and glucose, in this order, from majority to minority. The fatty acids profile mainly comprised linoleic acid (the majority), α-linolenic acid, and palmitic acid in all samples. AP presented also oleic acid (C18:1n9c), and eicosatrienoic acid (C20:3n3). Concerning organic acids, malic and oxalic acid were identified in all samples, with malic acid being the majority one. Citric acid was found in low quantities in IA. The principal phenolic compounds identified were phlorizin-3-glucoside, quercetin-3-*O*-rutinoside, and quercetin-3-galactoside. Regarding the TBARS assay, average IC_{50} values of 2.2 mg/mL were obtained for AP and 0.88 mg/mL for IA and for OxHLIA, values of 0.16 mg/mL for AP and 1.2 mg/mL for IA. Although the results are very different, they allow inferences about the mechanism of action of the extracts under study. Overall, the most notorious results are the large amount of dietary fibers and proteins that are present in AP, as well as the presence of polyunsaturated fatty acids and phenolic compounds. Thereby, the bio-residue obtained from apple processing has a high potential to be exploited as an innovative and competitive source of bioactive compounds, with potential applications in the food and nutraceutical industry. These applications withstand a highly promising way of adding value to apple pomace, for instance by reducing environmental impact and contributing to a circular economy.

F 020 PRECISION NUTRITION TO IMPROVE CARDIOMETABOLIC HEALTH WITH DIETARY (POLY)PHENOLS (PRE-CARE-DIET): A RESEARCH PROTOCOL

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Background:

Prevalence of cardiometabolic diseases is rising in the human population and a drastic change to plant-based dietary patterns, which are rich in bioactive phytochemicals, is advised. However, the heterogeneity in the metabolism of plant bioactives and individual responses to their ingestion deserve special consideration. In the case of (poly)phenols, these differences may result in several excretive phenolic profiles, which can be clustered to define phenolic metabotypes. Metabolic phenotyping according to (poly)phenol metabolites may pave the way for the development of targeted nutrition strategies to lower cardiometabolic disease risk with dietary (poly)phenols.

Objective:

The primary objectives of the PRE-CARE-DIET study will be assessing how a differential capacity to metabolize dietary (poly)phenols affects cardiometabolic health, and understanding the key determinants behind the heterogeneity in the individual's biological response to their consumption, adopting a comprehensive multi-omics approach and creating predictive models embracing the singularities of each individual.

Methods:

A 24-week, randomized, controlled trial will be conducted in 300 adults prospectively recruited according to their aggregated phenolic metabotype (Figure 1), determined through an *oral (poly)phenol challenge test*. Subjects (40–79 y.o.) will present at least one cardiometabolic risk factor and will belong to the 2 most prevalent aggregated phenolic metabotypes. The study includes an observational phase (8 weeks) where participants will follow their habitual diet. This phase will also serve as a run-in for the experimental phase (16 weeks), which follows a 4-arm design (Figure 1). Participants belonging to each metabotype (n=150 per metabotype) will be randomly allocated (2:1) into two groups: the *treatment arm* in each metabotype (n=100 per metabotype) consists of a 50% increase in the (poly)phenol intake of each individual; no changes in the (poly)phenol intake will be requested for one-third (n=50) of the subjects in each metabotype (*control arm*). The increase in the mean daily consumption of (poly)phenols will be delivered considering the intake in total (poly)phenols of each subject and his/her preferences, as a personalised intervention. Biological samples will be collected and used to perform targeted and untargeted metabolomics, gut and oral microbiome profiling at species level, genetic polymorphisms (SNPs) profiling, and the comprehensive assessment of several panels of biomarkers related to cardiometabolic risk, intestinal permeability, adipose tissue function, and food intake regulation. Personal, anthropometric, body composition, dietary and lifestyle data will also be collected. The primary outcome is the 10-year risk of cardiovascular disease (SCORE2 and SCORE2-OP).

Expected results:

This study will unravel how a differential capacity to metabolize (poly)phenols affects cardiometabolic health outcomes, while considering individual's heterogeneity related to preferences, microbiome, and genotype. The results of this study will contribute to the development of a new way of managing chronic diseases, fully individualized and based on multi-omics techniques, prediction algorithms, and plant-based diets with proven health benefits.

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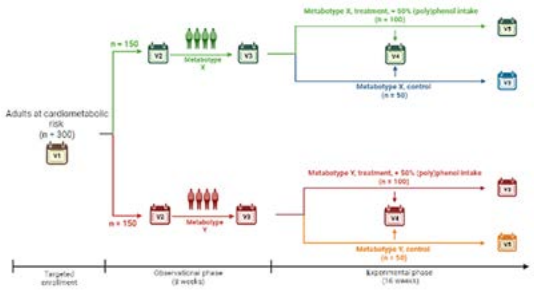
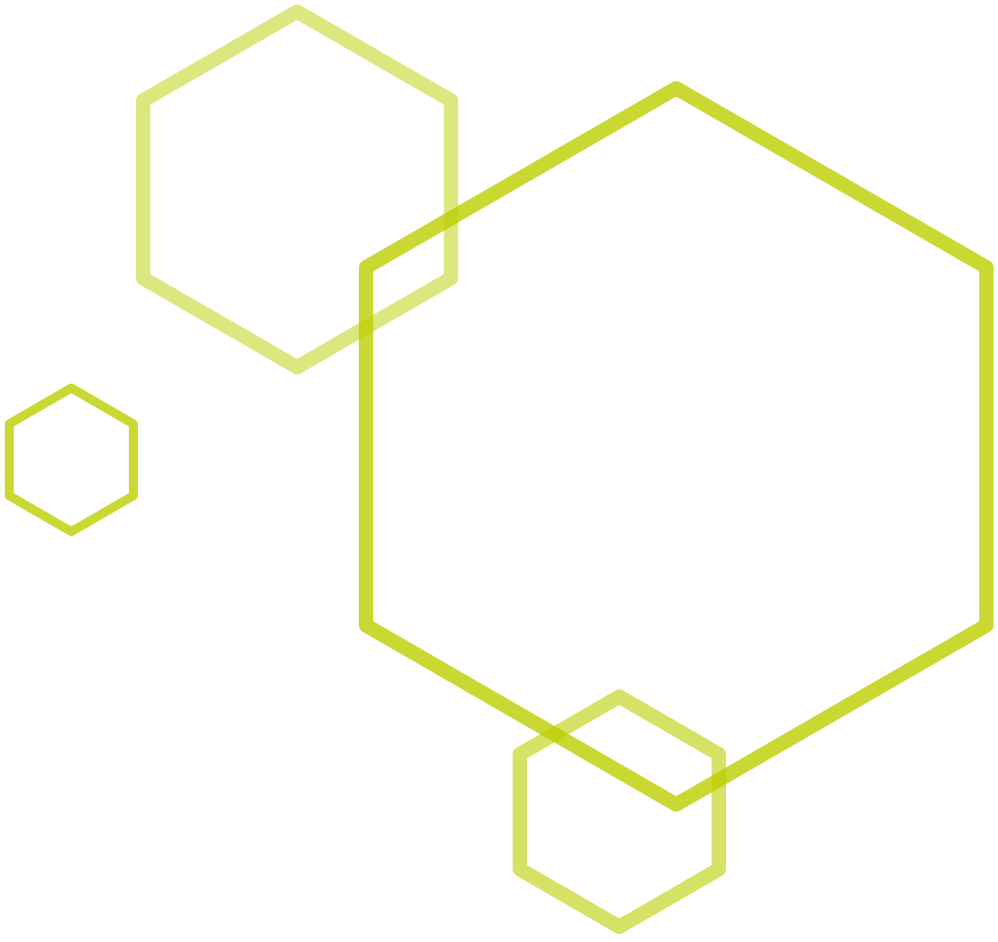


Poster Presentations



P 001 PHENOLIC METABOLITES IN ANIMAL MILK: A NEW GATE FOR THE INTAKE OF PLANT BIOACTIVES?

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Background:

Milk is the biological fluid secreted by female mammals consisting of water, lipids, carbohydrates, proteins, vitamins, minerals, and, among other secondary compounds, a variety of (poly)phenolic metabolites. This composition is influenced by numerous factors, including genetics, breed, age, health status, lactation stage, and the animal diet. In this regard, the animal diet can be designed to modulate the composition and nutritional profile of milk, increasing the content of certain nutrients, and perhaps meeting the needs of consumers who desire healthy and sustainable dairy products. Thereby, isoflavones and lignans, as the predominant phenolic compounds in forage, and other (poly)phenols and their derived metabolites would be candidate compounds to be identified in milk. However, the literature related to the phenolic composition of milk is scarce.

Objectives:

The aim of this work was to assess the phenolic profile of different commercial milk samples, based on different types of animal, sterilization treatments, and fat and lactose contents.

Methods:

Thirteen milk samples found on the Italian market were analyzed. The types of milk studied were: whole and semi-skimmed pasteurized cow milk (two different brands each one); whole, semi-skimmed and lactose-free UHT cow milk (two different brands each one); semi-skimmed microfiltered cow milk; and whole and semi-skimmed UHT goat milk. An ultrahigh-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS) was used to profile the different milk samples.

Results:

The analysis of the different milks evidenced the occurrence of 23 diverse phenolic metabolites related to the metabolism of isoflavones, flavan-3-ols, lignans, and ellagitannins, among other families. The identification of different colonic metabolites in milk, such as equol, urolithins, and phenyl-γ-valerolactones, was of great relevance, as not all individuals are able to produce them. Regarding the concentration of phenolic metabolites, no relationship was established with the different types of milk regarding to sterilization treatment, fat, and lactose content. However, it is remarkable that, except for lignan metabolites, goat milk exhibited a higher concentration of phenolic metabolites in comparison to cow milk.

Conclusion:

The supplementation of animals with (poly)phenols generating these bioactive metabolites may increase metabolite concentration in milk, providing metabolites to individuals who are unable to produce them through daily consumption of milk. In this sense, individuals would consume these metabolites more regularly, as many of them are originated from (poly)phenolic compounds present in plant-based foods that are consumed sporadically. Moreover, increasing the consumption of goat milk could be a valuable strategy for increasing the consumption of (poly)phenol metabolites, as goat milk presents a higher quantity of them than cow milk for almost all metabolite families. Milk can be a new source of bioactive phenolic metabolites, but much work is needed before supporting animal milk as a significant contributor to the intake of bioactive phenolics.

P 002 A COMPARISON OF THE EFFECTS OF GREEN TEA AND COCOA ON GLYCAEMIC CONTROL AND INSULIN SENSITIVITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background:

Type 2 diabetes mellitus is among the most frequent chronic diseases in virtually all countries, and they pose a global health threat. Therefore, Preventive interventions are critical for reducing the enormous burden of diabetes. Flavanols may assist people with metabolic disorders to maintain glucose homeostasis by inhibiting digestive enzymes and glucose transporters. GT and cocoa have gotten a lot of attention since they›re high in flavanols.

Objectives:

To investigate the effects of polyphenol supplementation (GT and cocoa) on reducing the incidence of complications and improving glycaemic control and insulin sensitivity in adult patients with T2DM through outcomes FBG, FBI, HbA1c and HOMA-IR.

Methodology:

An electronic research programme was applied, using different databases (including Web of Science, PubMed/Medline, Scopus, and Cochrane Reviews) to find studies that have evaluated the impact of polyphenol supplementation (GT and cocoa) on reducing the incidence of complications and improving glycaemic control and insulin sensitivity in adult patients with T2DM. Articles published between 2005 and 2021 in English were selected.

Results:

A total of 12 and 7 studies involved 677 participants on GT (320 male/357 female), while the ones that focused on cocoa had 331 participants (83 male/ 248 female) with T2DM aged 18–65 years and a BMI range of 25–34.9 kg/m² were identified and included in the present review. The majority of the studies were fair-quality studies. The results of the meta-analysis showed no significant difference for FBG levels following GT but a highly suggestive significant difference for FBG levels following cocoa consumption compared to the control group (SMD: 0.121; 95% CI:-2.150–2.391; P=0.917 and SMD:-1.313; 95% CI,-2.378–0.249; P=0.016, respectively). The results showed no significant difference for GT and cocoa intervention (SMD: 0.549; 95% CI:-2.167–3.266; P=0.692 and SMD:-1.101; 95% CI:-2.813–-0.611; P=0.207, respectively). The results of the HbA1c analysis showed no significant reduction after GT consumption (SMD:-0.157; 95% CI: -0.408–0.094; P=0.221), but there was a statistical difference after cocoa consumption (SMD,-0.601; 95% CI,-1.131–-0.071; P=0.08). There was no evidence that GT interventions were effective in improving HOMA-IR (SMD: 0.254; 95% CI:-1.188–1.695; P=0.730).

Conclusion:

The evidence shows clearly that the short-term administration of cocoa reduced FBG and HbA1c but the reduction in FBI, and HOMA-IR is clinically limited. In addition, GT intake did not significantly reduce the level of FBG, FBI, HbA1c, and HOMA-IR.

Keywords:

Cocoa; Green tea; Dark chocolate; Catechin; Glycaemic control; Diabetic patients; Meta-analysis

P 006 EFFECT OF BOILING WATER TREATMENT ON THE PHENOLIC COMPOSITION OF OAK ACORN FLOUR EXTRACTS

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The European oak (*Quercus robur* L.) is one of the most significant indigenous species in European forests. The recovery of Pedunculate oak acorns as a food resource has recently gained momentum, supported by the adherence to a low carbon footprint lifestyle and the fruit›s nutritional value that has been reported [1]. Nevertheless, from a nutraceutical standpoint, this fruit potential can be expanded, particularly given how little research has been conducted.

The objective of this work was to study the effect of the treatment with boiling water on the phenolic composition of *Q. robur* acorn flour extracts. For this, one sample of oak acorn flour was treated with boiling water and another sample untreated was used as a control. Both were dried at 75 ºC before the obtention of the extracts. A solid-liquid extraction with water at 40 ºC for 60 min was employed to obtain the extracts, which were analyzed by HPLD-DAD-ESI/MS to quantify the phenolic compounds [2]. The antioxidant activity was determined using the TBARS method [3].

The total content of phenolic compounds was 42±1 mg/g of extract for the treated sample and 16.2±0.4 mg/g of extract for the control. Gallic acid got the greatest concentration in both samples, reaching values of 37±1 mg/g of extract and 10.8±0.4 mg/g of extract for treated sample and control, respectively. Ellagic acid and its derivatives (ellagitannins) were also found in the extracts and the sum of these compounds was 5.1706±0.0061 mg/g of extract for control and 5.179±0.123 mg/g of extract for treated sample. The results obtained for the antioxidant activity were 7.06±0.03 µg/mL and 10.15±0.05 µg/mL relative to control and treated sample, respectively.

The treatment applied to the sample appears to have impacted its antioxidant capacity, as observed from the results. Analysis of the phenolic composition revealed that both samples shared a similar profile of phenolic compounds. While the treatment negatively affected the sample, it did not alter the phenolic composition of either sample. However, additional research is required to identify the specific compounds that may be influencing the antioxidant capacity of the samples.

Acknowledgments:

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P 007 INFLUENCE OF DIFFERENT HARVESTING LOCATIONS ON THE PHENOLIC COMPOSITION OF *Quercus rotundifolia* LAM. ACORN FLOUR EXTRACTS

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The holm oak (*Quercus rotundifolia* Lam.) is significant in the Iberian Peninsula for its ecological, economic, and cultural value, as well as its contributions to climate change mitigation and rural development [1]. Exploring the acorn, a relatively unexplored fruit of this species, can enhance its chemical potential. The phenolic profile of plants, including holm oaks, can vary based on factors like location, climate, soil, genetics, and interactions with other organisms.

This work objective was to study the phenolic composition of *Q. rotundifolia* acorn flour extracts associated to the different location in which acorns were collected. For this, two samples were collected from different regions of Portugal: Avis, Portalegre, south of Portugal (sample 1) and Mogadouro, Bragança, North of Portugal (sample 2). A solid-liquid extraction with water at 40 ºC for 60 min was carried out to obtain the extracts. Phenolic compounds were analyzed by HPLD-DAD-ESI/MS [2]. The antioxidant activity of the extracts was performed using the TBARS method [3].

As expected, gallic acid and ellagic acid were the main phenolic compounds found in the samples. The total phenolic content was nearly three times higher in Avis samples compared to Mogadouro samples, with values of 1.51±0.01 mg/g and 0.52±0.01 mg/g of extract, respectively. Gallic acid was present in both samples, with concentrations of 0.272±0.003 mg/g and 0.515±0.007 mg/g of extract for Avis and Mogadouro acorns, respectively. Traces of gallic acid derivatives, like digalloyl-hexoxide-isomer-I and galloyl-HHDP-glucose, were also found in both samples. However, ellagic acid was only detected in the extracts from Avis acorns, with a concentration of 1.243±0.004 mg/g of extract. The results obtained for the antioxidant activity, expressed as EC_{50}, were 29.7±0.4 µg/mL and 43.0±0.5 µg/mL relative to Avis and Mogadouro origin, respectively. As expected, the sample with the highest concentration of phenolic compounds reached the lowest EC_{50} value.

In conclusion, both samples showed a similar profile of phenolic compounds. However, the Avis sample exhibited a significantly higher concentration of phenolic compounds compared to the Mogadouro sample. Additionally, only the Avis sample contained ellagic acid. Comprehensive studies considering various factors are needed to fully comprehend the impact of the harvesting location on the phenolic composition of holm oak acorns.

Acknowledgments:

This work was financed by "La Caixa" Foundation through "Programa Promove (Concurso 2020 - Projetos piloto inovadores)", within the scope of the project Acorn4MED- Valorização do resíduo florestal bolota para aplicação em biomedicina.

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P 008 THE ACUTE EFFECTS OF FLAVANOLS AND ANTHOCYANINS ON BLOOD PRESSURE AND COGNITIVE PERFORMANCE IN HEALTHY YOUNG ADULTS

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Background:

Diets rich in plant-based foods have been associated with both cognitive and physiological benefits in humans. These effects have been attributed to a range of different macro- and micronutrients including their high flavonoid content. Often, plant-based foods contain multiple groups of flavonoid compounds making it difficult to determine the relative contribution of specific flavonoids to observed effects.

Objectives:

This pilot study investigated the relative cognitive and blood pressure effects of two common flavonoid groups: anthocyanins and flavanols isolated in extracts of black rice and green tea, respectively. Doses were chosen to mimic those readily obtainable from a single portion of commonly consumed foods such as berries, chocolate, or a cup of tea.

Methods:

Fifteen young adult participants were supplemented with black rice extract containing 250 mg anthocyanins, green tea extract containing 90 mg flavanols, and a placebo, in a three-armed crossover study with a one-week washout period between each intervention. Blood pressure measurements (systolic and diastolic) and a cognitive measure of attention (using a simple reaction time task, SRT) were recorded at baseline and at two-hours after consuming each treatment in capsule form.

Results:

Data were analysed using a linear mixed model with baseline measurements included as a covariate. A treatment-related trend was observed for diastolic blood pressure, with lower values observed following consumption of the anthocyanin-rich black rice extract compared with the placebo ($p<0.10$). No blood pressure effect was observed for the flavanol-rich green tea extract. No significant cognitive effect of anthocyanins or flavanols was observed.

Conclusions:

Anthocyanins may be effective in lowering blood pressure. Similar peripheral vascular effects have previously been associated with benefits to cognition, via mechanisms of enhanced cerebral blood flow. However, improvements to SRT performance were not observed here. The small sample size and relative simplicity of the cognitive task may provide possible explanations for the lack of any observed cognitive effect. A larger sample size and a wider range of more cognitively demanding tasks are recommended for further investigation of these flavonoid extracts, particularly black rice extract which has shown potential here.

P 009 ASSESSMENT OF THE *IN VITRO* BIOACCESSIBILITY OF POLYPHENOLS FROM CHESTNUT FLOWER EXTRACT: A STEP TOWARDS EXPLORING ITS FUNCTIONAL POTENTIAL

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The valorisation of agro-industrial residues from the chestnut (*Castanea sativa* Mill.) production chain as a source of natural biomolecules offers a promising strategy to produce high-added-value ingredients while minimizing the environmental impact of this agrifood sector. Studies have indicated the bioactive and technological properties of polyphenol-rich extracts derived from chestnut by-products, highlighting their potential to be used as natural preservatives and functional agents in beverages. However, in order to exert health-promoting activities, phenolic compounds must first be bioaccessible, and the bioaccessibility of these compounds from chestnut extracts following gastrointestinal digestion remains largely unexplored. Therefore, the objective of this study was to evaluate the *in vitro* bioaccessibility and digestive stability of polyphenols from a chestnut flower extract intended to be incorporated into beverages. Chestnut male flowers were collected from a chestnut orchard located in the Northeast of Portugal and subjected to a semi-industrial extraction procedure by forced percolation using 80% ethanol (v/v) as the extraction solvent. The resulting phenolic-rich extract was concentrated, atomised in a spray dryer, and resuspended in two different concentrations for the experiment: 400 mg/L and 40 g/L, the former being used for its incorporation into prototypes of alcoholic and non-alcoholic beverages besides another higher concentration. Subsequently, *in vitro* digestion was performed using the internationally recognized INFOGEST static model. The phenolic compounds present in the initial extract, chyme, and bioaccessible fraction were analysed using HPLC-DAD-(ESI-)MS/MS after a cleanup step employing solid-phase extraction (SPE). A total of 28 different compounds were identified in the extracts, with the hydrolysed tannin chestanin being the predominant compound found (39.1±1.2 mg/g extract). Hydrolysable tannins constituted the primary class of phenolic compounds in the non-digested extracts, followed by flavonoids. The phenolic profile of the extract with a higher concentration remained unchanged after digestion, suggesting that the phenolic compounds surpassed the capacity of the static digestion method utilized, potentially due to tannins inhibiting the digestive enzymes. Conversely, in the extract concentration applied in beverage prototypes, both the chyme and bioaccessible fraction exhibited a distinct profile and a considerably lower quantity of phenolics compared to the non-digested sample. These observations indicate that the digestive conditions led to compound degradation and instability, resulting in relatively low bioaccessibilities despite their release from the matrix is not a limiting factor. Nevertheless, flavonoid glucosides, especially quercetin derivatives, consistently exceeded the bioaccessibility of the other compounds, except for the cretanin, which presented the highest relative bioaccessibility (30%). The findings will provide valuable insights for the development of stable ingredients and food products enriched with bioaccessible bioactive compounds from chestnut by-products, to better retain their health effects and promote sustainable approaches within the food and beverage industry. Ongoing investigations are evaluating the bioaccessibility of these compounds incorporated in beers.

Acknowledgements:

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P 010 ASSESSMENT OF POTENTIAL ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF HYDROETHANOLIC EXTRACTS ORIGINATING FROM SELECTED SOLID RESIDUES OF THE ESSENTIAL OIL DISTILLATION PROCESS

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Medicinal and aromatic plants (MAPs) are potential sources of naturally occurring polyphenols that are highly valuable for the food sector, either as food ingredients or as constituents of active packaging. Their use in the essential oil industry, produces a considerable amount of solid residues (SR) during the distillation process that could be exploited as a natural source of bioactive compounds. Hence, the aim of this study was to investigate the antioxidant and antimicrobial properties of phenolic extracts derived from SR remaining after the essential oil process.

SR of five species: Greek oregano (*Origanum vulgare* ssp. *hirtum*), rosemary (*Rosmarinus officinalis*), spearmint (*Mentha spicata*), lemon balm (*Melissa officinalis*), and salvia (*Salvia fruticosa*) were extracted with ultrasound-assisted extraction with a hydroethanol mix (50 mL ethanol/100 mL).

Total phenolic content (TPC) and antioxidant activity based on the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical inhibition method were assessed in each extract. Additionally, the antimicrobial activity of the phenolic extracts against the following bacteria: *Escherichia coli, Salmonella* Typhimurium*, Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Bacillus licheniformis,* and *B. cereus* was evaluated in concentrations of 125–3000 mg/L, applied with the broth dilution method, in 96-well microplates.

Extracts were effective against gram-positive species. Rosemary and salvia extracts exhibited stronger antibacterial activities against all gram-positive species, even at a concentration of 750 mg/L for most of them, while extracts of spearmint and oregano were effective against fewer gram positives and only at the highest concentration used. Lemon balm extract didn't exhibit any inhibitory effect; however showed moderate antioxidant activity, along with spearmint. On the other hand, oregano exhibited the strongest antioxidant activity, followed by salvia and rosemary.

This research suggests a potential use of the studied extracts from SR as antimicrobial substrates in the food industry since they represent rich sources of bioactive compounds.

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P 011 POLYPHENOLS AND THEIR SULFATED DERIVATIVES AS MOLECULAR SIGNALING MODULATORS

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Several lines of evidence suggest that polyphenols can modulate oxidative stress and inflammation by inducing nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 has been identified as a master regulator of the transcription of antioxidant and many other cytoprotective genes. Polyphenols are the most abundant antioxidants in fruits, vegetables, spices, and beverages. However, after digestion, these compounds are converted into new metabolites, mainly conjugates, including sulfates. New functional groups can alter the properties of the molecule in relatively predictable ways. Sulfation, a reaction in phase II of the biotransformation of xenobiotics, increases the solubility of polyphenols and thus potentially their bioavailability. On the other hand, sulfated polyphenolic metabolites, as likely found after biotransformation, may not have the same effect on Nrf2 activation as non-sulfated parent compounds.

Luteolin and myricetin have a prooxidant effect and activate the nuclear factor Nrf2 by regulating the stability of the Keap1 protein. Proper interaction with the receptor requires a suitable complementary structure of the ligand, which means that the correct stereochemistry of the respective "antioxidant" plays a crucial role.

Using recombinant aryl-sulfotransferases[1] we have prepared mono- and disulfated derivatives of luteolin and myricetin. These flavonoids have already been published as Nrf2 activators[2]. The sulfated derivatives were enzymatically prepared, isolated, and structurally characterized. These compounds were then tested on retinal pigment epithelium (RPE) cells and their effects were compared with those of the non-sulfated polyphenols as Nrf2 activators. Sulfated metabolites may lose their "antioxidant" regulatory potential compared with non-sulfated flavonoids. On the other hand, their affinity and influence on the stability of KEAP1 and Nrf2 may be increased by sulfation, suggesting that the study of flavonoids as Nrf2 regulators is useful.

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P 013 COMPARATIVE STUDIES ON POLYPHENOLIC COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *Artocarpus altilis* (L.) LEAVES

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Breadfruit (*Artocarpus altilis* L.) is an abundant crop originating from the pacific islands, cultivated for over 3000 years in the Caribbean West Indies. Several studies have been published about breadfruit, but few have described its leaves. However, *Artocarpus altilis* (L.) leaves, which are largely underutilized, are increasingly being consumed in juice, for example. They are thought to have beneficial health effects but there is no research to confirm this.

This study presents a comparative analysis of the dietary and pharmacological compounds found in the leaves of *Artocarpus altilis* (L.) at two different stages of maturity: green and yellow. We performed analyses of total proteins, polyphenols and flavonoids contents in both types of leaves. Qualitative and quantitative analyses of the polyphenolic compounds were achieved using an HPLC-UV method. Biological properties such as antimicrobial and antiradical activities were studied.

The results indicate that the total protein content of the yellow leaves is significantly lower than that found in the green leaves. Conversely, the yellow leaves have significantly higher total polyphenol and flavonoid contents than the green leaves. Antioxidant activity tests were carried out on the leaves using the DPPH method, which revealed similar values for both types of leaves. These results suggest that yellow leaves may represent a richer source of phenolic compounds than green leaves, with potential applications as a natural source of antioxidants and other bioactive compounds. While the green leaves show a veryvery interesting total protein content compared to many other plant sources, suggesting they could be used to develop new protein sources. In the antimicrobial assay, *Artocarpus altilis* leaf extracts demonstrated efficient activities against bacteria.

Further analysis is necessary to determine the precise nature of the pharmacological compounds and biological properties, such as anti-cancer and anti-inflammatory activities, in *Artocarpus altilis* (L.) leaves at these two stages of maturity.

P 014 PHYSIOLOGICALLY RELEVANT CONCENTRATIONS OF QUERCETINS, CHLOROGENIC ACIDS, AND THEIR COLONIC METABOLITES INHIBIT THE PROLIFERATION OF TWO COLON ADENOCARCINOMA CELL LINES

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Several studies suggested that a phenolic-rich diet may be protective against the onset of colon cancer. Most of the phenolic compounds introduced with the diet are not absorbed in the small intestine and reach the colon where they can be metabolized by gut microbiota in simple phenolic acids. However, given the considerable interindividual variability in the composition of the human microbiota, the amount and type of metabolites produced differ greatly among individuals. Therefore, both progenitor compounds and microbial metabolites coexist in the colon and the ratio of progenitor compounds/metabolites depends strongly on the different colon human metabotypes.

In this study the anti-proliferative activity of dietary quercetins, chlorogenic acids, their colon metabolites and selected mixtures of parent compounds/metabolites was assessed by using two different colon cancer cell line models (Caco-2 and SW480) at physiologically relevant concentrations.

Human colon cancer cell lines Caco-2 and SW480 were cultured and grown. All phenolic compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mmol/L, diluted in the respective cell culture media at a concentration of 100 µmol/L. Cell proliferation was assessed by the MTS assay. Incubation was carried out for 24, 48, and 72 hours. The data were compared with a control solution containing 0.5% DMSO in the cell medium and representing 100% proliferation. For the most active compounds, IC_{50} values were calculated by performing the antiproliferative assay with different concentrations of the specific phenolic compounds (range 1.5 to 200 µmol/L). In addition, the analysis of the cell cycle and possible apoptosis was carried out using Nicoletti›s reagent. In this case, cells were seeded and treated with the different phenolic compounds at the concentration corresponding to the IC_{50}. After a 72-h incubation, the cells were centrifuged and resuspended in 0.5 ml of Nicoletti‘s solution. Finally, a cytofluorometer reading was taken after 15 min of incubation in the dark.

Chlorogenic acids, quercetin, and the metabolite 3-(3’,4’-dihydroxyphenyl)acetic acid were the most active against Caco-2 cells, whereas quercetin-derivatives, and the metabolites 3-(3‘-hydroxyphenyl)acetic acid and 3-(3’,4’-dihydroxyphenyl)acetic acid were the most active against SW480. 3-(3’,4’-dihydroxyphenyl)acetic acid was found to be the most active compound against proliferation of the Caco-2 cell line after 72 h of incubation (IC_{50}=3.0 ± 0.1 µmol/L), while quercetin was the compound that showed the highest antiproliferative activity against SW480 cell line (IC_{50}=58.3 ± 2.7 µmol/L).

The mixtures parent compounds/metabolites that mimic the colon human metabotypes that metabolize slowly or rapidly the parent compounds (low-producers vs high-producers) similarly inhibited cell growth.

SW480 cells metabolizes parent phenolic compounds more rapidly and extensively than Caco-2, whereas colon metabolites were generally more stable.

Finally, the cytofluorometer study showed that some compounds induced apoptosis and/or cell cycle modification in the two cell lines.

These results suggest that dietary phenolic compounds exert remarkable anti-proliferative effect against human colon cancer cells that can be further sustained by the produced colon metabolites which retained the anti-proliferative effect. Therefore, gut microbiota metabolism of phenolic compounds may be of paramount importance in explaining the protective effect of phenolic-rich foods against colon cancer.

P 015 EFFECT OF A POLYPHENOL RICH DIET ON MEMORY AND POLYPHENOLS DISTRIBUTION IN A CHICK MODEL

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Polyphenols are bioactive compounds abundant in plants that have the potential to exert a protective effect against neurodegeneration and enhance memory. A chicken model was used to study the effect of feeding purple corn or a red–berry mixture on polyphenols bioavailability and cognitive functions in chicks for 14 days. A total of 120 one day old male broiler Cobb chicks obtained from a commercial hatchery were housed in an experimental farm and allocated to 30 pens, each containing 4 chickens, to receive three different dietary interventions during 14 days. Three isonutritive corn/soya-based diets only differing in the polyphenol sources were formulated as following: 1) a yellow corn control diet (YC), 2) a Purple corn based diet (PC) where yellow corn was replaced for purple corn, and 3) a diet YC supplemented with 2% of red berry mixture (RB). Purple corn was a black corn variety called 'Millo Corvo' (Millo Corvo), and red-berry mixture contained a blend of red and black currant (16.7% and 33.3%, respectively), raspberry (33%) and blueberry (16.7%). Diets were offered to the birds (40 birds per diet) for 14 days and the bioavailability of dietary polyphenols and the effect on memory was studied following the chicken memory model developed in a previous experiment (Chamorro et al 2023). Chicks fed experimental diets were trained in avoidance learning task using methyl anthranilate, and the recall was tested at 24, 48 and 72 hours after training. At the end of the memory test (14 days of age) 10 birds per treatment were randomly selected, sacrificed and blood and brain of chickens were immediately collected, acidified, and used for the study of polyphenol bioavailability. The total polyphenol content was determined in diets and plasma by Folin-Ciocalteu, and the identification and quantification of plasma and brain metabolites was performed by HPLC-MSMS-QTOF. The total polyphenol content of the experimental diets was 0.54, 0.68 and 0.87 mg gallic acid equivalent/g for YC, PC and RB diets, respectively. Our results showed that birds fed polyphenol enriched diets tended to present a higher plasmatic total polyphenol content than those fed the other diets (by 18 and 11 % in those birds fed purple corn and redberry respectively). These results suggest that birds might digest and/or metabolize these polyphenolic sources. In this sense, the total polyphenol increase was correlated with a higher plasmatic content of several phenolic acids such as gallic acid and dihydroxybenzoic acid that were four and two-fold respectively higher in those birds fed polyphenol enriched diets. In chicks' brain, gallic acid and hydroxybenzoic acid were also detected, however surprisingly, the content of these phenolic acids was higher in the brain of birds fed the control diet. Regarding the effect on memory, no effect of feeding polyphenol sources on retention rate was observed. Our results suggest that these sources of polyphenols are bioavailable and reached the systemic circulation of birds however the capacity to be transferred to brain tissue and its role on memory remained unclear.

P 016 EFFECT OF DIETARY ADDITION OF GRAPE POMACE ON INTERNAL AND EXTERNAL EGG QUALITY

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Grapes and their associated by-products (as grape pomace, GP) are highlighted for their polyphenol content, a source of bioactive compounds with antioxidant capacity. The aim of this study is to determine if the addition of 5% of GP to hen diets might produce an enrichment in egg antioxidants and to study its effect on internal and external egg quality parameters. An experiment was conducted in 24 hybrid white leghorns hens which were divided into two groups: a control group fed with a control diet (without GP), and an experimental group fed with a 5% GP supplemented diet. Both diets were formulated to be isonutritive and only differing in their polyphenol content. The experiment lasted 6 weeks, and during the last 3 weeks eggs were daily collected, weighted and used to determine the internal quality (Haugh Units and yolk colour) on fresh eggs and on eggs stored in refrigeration for 15, 21 and 30 days. Haugh units, a measure of albumen quality, was calculated considering the height of thick albumen and the egg weight. The yolk color was measured using the roche yolk color fan, a 16 scales color index which allow to distinguish the yolk color density. The external quality was studied by measuring the eggshell thickness and the eggshell breaking strength using a texturometer. Eggs yolks of fresh eggs were separated, lyophilized, and used to determine retinol, α- and γ-tocopherol using HPLC with UV detection. Additionally, yolk samples were subjected to a methanolic extraction and analyzed by HPLC-MSMS-QTOF for metabolites identification. As expected, Haugh units decreased with time. The negative effect of storage on Haugh unit was reduced in the eggs obtained from hen fed GP supplemented diets. Regarding the external quality, dietary GP reduced eggshell thickness whereas no effect on breaking strength was observed. This effect on external egg quality could be related to the chelating ability of plant polyphenols that might have reduced the eggshell calcium content. Grape pomace did not modify the content of retinol, α- and γ-tocopherol when compared to the control group. In the yolk samples we identified the presence of gallic acid, being the content of this phenolic compound higher in the eggs of hen fed GP diets, suggesting that some dietary phenolic compounds could be transferred to the eggs. In conclusion dietary supplementation with GP improved the internal quality of fresh eggs up to 21 days of storage but worsened the shell quality.

P 017 OPTIMIZATION OF GREEN ACCELERATED SOLVENT EXTRACTION OF ROSMARINIC ACID, CARNOSOL AND CARNOSIC ACID IN *Rosmarinus officinalis* L. DISTILLED SOLID RESIDUES

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Nowadays, there is a growing interest in developing natural antioxidants by using ''green'' processes, that could be served as alternative to the synthetic antioxidants. Moreover, within the concept of sustainable use of the available resources there is a need to develop new strategies for making the best use of our resources and eliminating waste along the supply chain. There is an opportunity to make a sustainable profit from low-cost raw material. *Rosmarinus officinalis* L. (rosemary) is a well-known medicinal and aromatic plant, being a rich source of natural antioxidants, known for their beneficial health-promoting properties. Rosemary is mainly used as a culinary herb and for its essential oil, with the latter generating considerable amounts of solid residues (SR) during production, leading to serious environmental issues. However, SR contain exploitable natural sources of biologically active compounds, being of particular importance such as phenolic compounds. The present study aimed to optimize the ''green'' solvent extraction of rosemary distillation SR, and particularly the Accelerated Solvent Extraction (ASE) conditions in order to maximize the main phenolic compounds of rosemary extracts, based on Response Surface Methodology (RSM). A three-factor Central-Composite Design (CCD), consisting of a total of 15 experimental runs, was used to investigate the effects of three factors: solvent concentration (X1, ethanol 20–80%), time extraction (X2, 3–7 min under 3 cycles) and temperature extraction (X3, 65–125 °C) on the responses: extraction yield, rosmarinic acid (RMA), carnosol (CARO) and carnosic acid (CARA) contents. Phenolic compounds were extracted by the use of ASE (Dionex ASE 350, ThermoScientific) from dried SR of rosemary, under standard operation pressure at 1500 psi, using a flush volume of 65%, a purge time of 90 s and cell preheating time of 5 min. Rosemary ASE extracts were analyzed by HPLC-DAD-MS for quantification of RMA, CARO and CARA. Results indicate that an extraction temperature of 125 °C with 60% ethanol concentration for 7 min of automated extraction cycles, were optimal for the recovery of rosemary extracts rich in phenolic compounds. The effectiveness of ASE as a green extraction technique was compared to conventional methods e.g. the Soxhlet technique. The ASE produced a higher extraction yield of rosemary extract with a greater amount of CARO and CARA than the Soxhlet method, but a similar concentration of RMA. Furthermore, ABTS and DPPH free radical scavenging assays were used to investigate the antioxidant activity of the extracts. The present study indicated that the ASE method could be especially useful for extraction of highly concentrated and bioactive rosemary extracts that could be used in food, food packaging, pharmaceutical, perfumery and cosmetics industries, minimizing solvent consumption, energy, time and cost of the process compared to the classical methods. Moreover, ASE could be considered as a novel promising and fully automated extraction technique to be scaled up in the industry production.

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P 018 EXPLORING THE POTENTIAL OF THERMAL WATERS FOR ENHANCING BIOACTIVE PROPERTIES OF GERMAN CHAMOMILE INFUSIONS

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German chamomile (GC, *Matricaria recutita* L.) is a plant traditionally prepared as an infusion and used for medicinal purposes. Thermal waters (TW) are natural mineral waters with therapeutic applications. Studies have shown that the health-beneficial effects of TW are related to the chemical and physico-chemical features of the water source, such as the mineral profile and the temperature. This study aimed to investigate the effect of preparing GC infusions with TW rather than with drinking-water (DW) on the bioactive properties of GC extracts. TW from two different sources was used: TW from Chaves (86 °C in the source) and TW from Vimioso (17 °C in the source). The GC infusions were prepared by bringing TW from Vimioso and Chaves to boiling and leaving dried GC leaves to remain in infusion for 5 minutes; or by leaving GC leaves in TW from Chaves at 86 °C (temperature of the source without any further heating) for 5 minutes. The infusions were filtered, freeze-dried, and tested for phenolic compounds composition (PC), antioxidant, hepatoxicity, antimicrobial, anti-inflammatory, and anti-tumor activities. Infusions prepared with DW were used as a control. PC were determined using LC-DAD-ESI-MS^n, antioxidant activity was analyzed through a chemical method analyzing the radical scavenging capacity (DPPH) and a biochemical method that measures the lipid peroxidation inhibition (TBARS), while the remaining bioactivities were evaluated using cell-based assays. The major phenolic compounds tentatively identified in the samples included ferulic acid hexoside, apigenin-*O*-malonylhexoside, apigenin-*O*-hexoside, caffeoylquinic, and dicaffeoylquinic acid isomers. The TW (from Vimioso or Chaves) used for infusion preparation showed an effect on the PC profile, with TW from Vimioso showing a greater number of compounds and a higher concentration of them. Moreover, infusions prepared with TW from Chaves after boiling showed a higher concentration of PC compared to infusions made with TW at the temperature in the source (86 °C). In general, the extracts showed similar antimicrobial activity against Gram-positive and Gram-negative bacteria of food interest, such as *Salmonella enterica, Bacillus cereus,* and *Staphylococcus aureus*, and clinical interest, namely *Klebsiella pneumonia*, *Escherichia coli, Morganella morganii.* The minimal inhibitory concentration (MIC) was between 5 and 10 mg/mL for all the extracts, which was much lower than the tested positive control. For the antifungal activity against *Aspergilus brasiliensis* and *Aspergilus fumigatos*, the MIC was lower in extracts prepared with TW from Chaves (0.6 mg/ml) versus 2.5 mg/mL of extracts obtained with TW from Vimioso, and 5 mg/mL of those prepared with DW, suggesting a potential synergistic effect between TW from Chaves and GC. None of the samples showed hepatoxicity. With regard to the anti-inflammatory and anti-tumor activities, they were not significant within the concentration range tested (until 400 mg/mL). All the samples showed antioxidant activity by the used methods. Therefore, the results indicate the potential benefits of preparing traditional infusions with TW to improve their biological properties.

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P 019 CYTOTOXICITY AND ANTIOXIDANT ACTIVITY OF BY-PRODUCTS FROM BRAZILIAN COFFEE PRODUCTION

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Background:

Coffee has been gaining prominence in recent years not only for its flavor, but also for being an important source of antioxidants, generating positive results for the health of its consumer public. Its beneficial health effects are due to the presence of bioactive compounds, highlighting phenolic compounds, alkaloids and some other compounds that have such benefits, that can contribute to the reduction and/or prevention of many chronic diseases. Defective and/ or green coffee beans are fruits that have not reached the desirable maturation stage or that have some aspect different from the beans considered healthy due to errors in harvesting, post-harvesting, processing or even genetic or physiological anomalies. Because of this, these fruits are little used by the coffee industry and are, therefore, considered as by-products of coffee production. Its chemical composition is complex and may vary according to the species, grain variety, climate, soil, altitude and cultivation methods. Faced with the growing consumer demand for healthiness and sustainability in food production chains, obtaining bioactive compounds from by-products of coffee production, such as green and/ or defective coffee, becomes a great opportunity to be explored.

Objectives:

Based on the exposed, the aim of this work is to determine the phenolic composition and its antioxidant capacity of the defective and/ or green coffee and to evaluate its cytotoxicity, to determine whether its use as a food ingredient would be feasible.

Methods:

A hydroalcoholic extraction of defective and/ or green coffee was made. The characterization of the phenolic profile was carried out through chromatographic analysis by high-performance liquid chromatography (HPLC) and total phenolics analysis by Folin-Ciocalteau method. An analysis of *in vitro* antioxidant capacity was also performed using ORAC tests and quantification of reactive oxygen species (ROS) in AAPH-induced Caco-2 intestinal cells. Cytotoxicity was also measured in Caco-2 cells, through MTT assay.

Results:

The phenolic profile show that the hydroalcoholic extract from defective and/ or green coffee contains approximately 133.2 ± 3.6 µg of gallic acid/ mg of lyophilized extract, being the major compounds: chlorogenic acid (239 mg/ g of lyophilized extract), caffeine (43.1 mg/g) and epicatechin (17.4 mg/g). The results of the antioxidant capacity showed that the lyophilized extract has an ORAC activity of 2.96 mM of Trolox equivalent. The cytotoxicity of the extract was tested in different concentration range (1000–0.1 µg/mL) and none of the concentrations analyzed showed cytotoxicity. Concentrations between 250–5 µg/mL showed an antioxidant activity in Caco-2 cells induced with AAPH, being the concentration of 50 µg/mL the most efficient, with reductions of up to 65%.

Conclusion:

The hydroalcoholic extract of defective and/ or green coffee showed no cytotoxicity and great potential for antioxidants in foods products.

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P 020 STUDY OF THE IMPACT OF *IN VITRO* DIGESTION ON POLYPHENOLIC COMPOUNDS IN BLACK BEANS (*Phaseolus vulgaris* L. CV. *tolosa*) BASED PRODUCTS

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Black beans (*Phaseolus vulgaris* L.) are globally consumed for their nutritional and functional properties, including the health benefits derived from their polyphenolic composition. However, processing methods utilized to achieve desired consumption forms, as well as improved taste and appearance, can impact the bioaccessibility of nutrients. During digestion, antioxidant compounds undergo structural modifications that can affect their absorption and bioactivity. This study aimed to investigate the effect of *in vitro* digestion on the antioxidant compounds present in black bean (*Phaseolus vulgaris* L. cv. *Tolosa*)-based products. Samples of cooked black beans, hummus, and black bean flour (as a control) were digested using the INFOGEST protocol. Total phenolic content (TPC, Folin-Ciocalteu), antioxidant activity (by FRAP and DPPH), and HPLC-DAD/MS-MS were evaluated before and after digestion. Besides, the resulting digested samples were then used in the trans-well model with *caco-2* cells. Compared to the control, both processing methods led to significant increases ($p < 0.05$) in TPC. Cooked black beans exhibited 10.4 mg gallic acid equivalents (GAE)/g DW, while hummus showed 4.6 mg GAE/g DW, in contrast to the control with 1.4 mg GAE/g DW. Regarding ferric reducing power (FRAP), at the end of the digestion, cooked black beans demonstrated a higher value of 14.3 µmol Trolox eq/g DW compared to the control with 10.8 µmol Trolox eq/g DW. In contrast, hummus presented a significant decrement in FRAP activity, recording 7.5 µmol Trolox eq/g DW. Similar trends were observed for antiradical activity (DPPH), with cooked black beans showing a 26% increase (11.0 µmol Trolox eq/g DW) and hummus displaying a 47% decrease (4.7 µmol Trolox eq/g DW) relative to the control (8.8 µmol Trolox eq/g DW). HPLC-DAD/MS-MS analysis revealed the presence of three main anthocyanins before digestion, pelargonidin-3,5-O-(di glucoside), cyanidin-3-O-glucoside, and pelargonidin-3-O-(glucoside). However, these compounds disappeared at the end of the digestion of the black bean-based products while they persisted in the control. Furthermore, the *caco-2* model exhibited a protective effect, as black bean products demonstrated significantly increased cellular viability compared to the control, with cooked beans showing a 72 % increment and hummus a 117 % increment. In summary, when beans are cooked, bioaccessible polyphenols and their antioxidant activity increase, which also has a positive effect on cell viability. When cooked beans are processed into hummus, although TPC increases and antioxidant activity decreases, it still has a protective effect. Overall, black bean-based foods retain bioaccessible and bioactive polyphenols after different processing and *in vitro* digestion phases, despite the loss of main anthocyanins during digestion.

P 021 PROTECTION OF PHENOLIC COMPOUNDS CO-ADMINISTERED WITH INULIN UNDER *IN VITRO* GASTROINTESTINAL CONDITIONS

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The extensive exploitation of the olive tree in the Mediterranean area has led to the accumulation of an abundance of associated by-products, prompting environmental concerns for their elimination methods and the search for innovative processing alternatives. Therefore, revalorization of these by-products has been proposed to promote a circular economy approach in this sector and for the development of high added-value products from related industries.

Among these by-products, olive leaves have emerged as a novel source of bioactive molecules, particularly phenolic compounds. Phenolic-rich derived extracts from this source have proved to exert an abundance of bioactive properties, from antioxidant, antimicrobial and antiviral activities. Additionally, they have also been implicated in pathologies such as obesity and several gastrointestinal disorders, with recent research highlighting their relation to their interaction with colonic microbiota. However, in order to observe their beneficial effects these bioactive compounds need to reach the areas of interest which may be hindered by their instability under gastrointestinal conditions, limiting their applicability.

As a result, a new interest has been found on the evaluation of different strategies focused on the improvement of this critical aspect on polyphenols' bioaccessibility, as an essential factor for their use. In this regard, the influence of the upper gastrointestinal conditions on the phenolic profile of an olive leaf extract enriched in oleuropein and hydroxytyrosol co-administered with inulin, a prebiotic carbohydrate, was examined through the evaluation of the bioaccessibility of its main bioactive compounds. The co-administered formulation was submitted to an *in vitro* gastrointestinal digestion process following the INFOGEST 2.0 method. Phenolic content in both bioaccesible and residual fractions of the digestates were qualitatively and quantitatively analyzed at the end of the gastric phase and at different time points of the intestinal phase by HPLC-ESI-TOF-MS.

Bioaccesibility of the evaluated phenolic compounds showed a reduction for most compounds during the gastric phase, with an increase in content during the intestinal phase, remaining stable at high values throughout the mentioned stage. Behaviour under gastric conditions correlated with a higher presence in the residual phase and a later reduction for the following stage. As for the most abundant phenolic compounds, oleuropein showed a high recovery during the digestive process, with a high bioaccesibility at the end of the intestinal phase. However, highest bioaccesibility was found for hydroxytyrosol glucoside, related to oleuropein degradation. The presented results suggest an improvement on bioaccesibility of olive phenolic compounds when co-administered with inulin as an indigestible prebiotic fibre compared to previous literature. This could open a new opportunity for the development of innovative formulations with increased stability under gastrointestinal conditions.

P 022 PRE-CLINICAL STUDY OF OXYRESVERATROL AND ASSOCIATION WITH DOXORUBICIN IN A MURINE BREAST CANCER MODEL

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Background:

Breast carcinoma affects a large part of the female population, characterizing an important public health problem worldwide. Studies demonstrate that research methods for breast cancer using murine models are fundamental to understanding tumor progression and therapeutic response to anticancer drugs. In this context, research on antineoplastic substances based on natural products associated with experimental models is increasingly important in the search for new classes of chemotherapeutic agents. It is known that phytochemicals act by preventing the process of tumor formation, with reduced toxicity when compared to conventional therapy. Oxyresveratrol (ORV) is a polyphenol found in blackberry (*Morus nigra*) and has biological effects such as antioxidant and anti-inflammatory activities, considered underlying mechanisms of various pharmacological properties. However, little is known about its effects on breast cancer. Objectives: The aim of this study was to investigate the effect of ORV and chemotherapeutic Doxorubicin (DOX), isolated and associated in 67NR murine breast cancer cells, *in vitro* and *in vivo*.

Methods:

The viability of 67NR cells was evaluated using the MTT assay. The phases of the cell cycle were analyzed in flow cytometry after labeling with RNAse-propidium iodide (PI). The characterization of cell death was investigated by labeling with PI and Annexin-V. The murine breast cancer cell line 67NR was inoculated subcutaneously into the left fourth mammary fat pad of BalB/c mice, and after establishing the tumor mass, the animals were treated with ORV at a dose of 50 mg/Kg intraperitoneally. Results: Our results indicated that ORV, in different concentrations exerts cytotoxicity after 24, 48 and 72 hours in 67NR cells with IC50 values of 118.40, 57.71 e 36.24 µM, respectively. The association of the ORV reduced the cell viability in a dose dependent manner with decrease of 16-fold the concentrations of DOX. Cell cycle analysis demonstrated a significant increase in the sub-G0/G1 phase in 6.36 and 8.37-fold, after treatment with ORV and association for 24 hours, respectively. In addition, PI and Annexin-V showed that association increased annexin-V/PI-positive cells ratio by 4.27-fold after treatment for 24 hours, indicating late apoptosis. Our *in vivo* results demonstrated a significant reduction in the weight of primary tumors in the ORV-treated group after 35 days. In addition, we observed that the characteristics of the tumor mass were heterogeneous between the groups, since the tumors of the animals treated with 50 mg/Kg of ORV were smaller, more rounded and delimited and with the absence of blood vessels, when compared to the control group. Furthermore, our results demonstrate that there was no significant change in serum alkaline phosphatase and creatinine K values, and there was a 1.3 and 4.5-fold reduction in serum TGP and urea levels in the ORV-treated group, respectively.

Conclusion:

Our results evidence the *in vitro* and *in vivo* anti-breast cancer effect of ORV, which may suggest an important source of new molecules to support anticancer therapy.

P 023 APPLE JELLY ADDED WITH JABOTICABA POWDER: FROM PHENOLIC CONTENT TO METABOTYPES IDENTIFICATION AND CYTOTOXICITY IN BREAST TUMOR CELL LINE

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Jaboticaba (*Myrciaria jaboticaba*) is a Brazilian berry, which presents high phenolic compounds content, mainly anthocyanins and ellagitannins, phytochemicals present mainly in the peel and seeds, portions that are not consumed. For several classes of polyphenols, metabotypes have been identified, based on individual's capacity to produce specific metabolites. Studies describe three urolithin metabotypes in the population, metabotype A (urolithin-A producers), metabotype B (urolithin-A and isourolithin and/or urolithin-B producers) and metabotype 0 (non-producers of these urolithins), this distribution is associated with factors such as intestinal microbiota composition and nutritional status. The objective was analyze phenolic content (PC) and antioxidant activity (AA) before and after *in vitro* simulated digestion and investigate the urolithin metabotypes and excretion in volunteers who made acute consumption of an apple jelly prepared with jaboticaba peel and seed powder (AJJP). Additionally, we verified whether the AJJP would be able to present a cytotoxic effect on breast cancer cells. Antioxidant capacities were determined by FRAP and TEAC assays, and the quantification of PC in AJJP and urine by Fast Blue method. INFOGEST protocol was used to *in vitro* simulating gastrointestinal digestion. Metabolites in urine by HPLC/MS. For the cytotoxicity the MTT assay was performed. The addition of JP in jelly increased total PC by 2.65x (from 83.8 ± 8.0 to 222.0 ± 9.2 mg GA/mL). Our results showed the antioxidant activities by FRAP and TEAC of AJJP were 11943.2 ± 243.8 µM Fe_2SO_4/g and 4796.0 ± 350.3 µM Trolox/mL, respectively. After the AJJP *in vitro* simulated digestion process, the oral phase obtained greater antioxidant capacity by the FRAP and TEAC methods with 7849.6 ± 955.6 µM Fe_2SO_4/g and 36872.0 ± 2203.0 µM Trolox/mL, followed by the gastric phase with 4138.5 ± 346.9 µM Fe_2SO_4/g and 29594.0 ± 3747.0 µM Trolox/mL and intestinal phase with 1194.1 ± 543.1 µM Fe_2SO_4/g and 26928.0 ± 419.4 µM Trolox/mL, respectively. For PC, the oral phase had higher levels, followed by the gastric and intestinal phases, with 3074.0 ± 207.4, 1690.0 ± 131.9 and 1690.0 ± 84.8 mg GA/mL, respectively. After consumption of AJJP (containing 35.2 mg of vescalagin + ellagic acid), 24 h-urine samples were collected and total PC varied from 104.0 ± 1.4 to 450.3 ± 2.7 mcg GA/mL. We also identified three metabolites in urines: urolithin A 3/8-glucuronide, isourolithin A 3-glucuronide and urolithin B-glucuronide. The amount of total urolithins excreted by the volunteers ranged from 3.66 to 10.11 µmol/ 24 hours. Considering all volunteers (n=6) the prevalence was 33.3% of metabotype A, 50% of metabotype B and 16.7% of metabotype 0 individuals. For cytotoxicity assay in MDA-MB-231 cells, the AJJP extract obtained an IC_{50} of 751.67 ± 40.27 µg/mL, after 48 hours of treatment. In conclusion, AJJP showed high PC that enhanced after simulated *in vitro* digestion. For the AA, the results were contrasting depending on the method utilized, having TEAC a greater correlation with the PC. Metabotype B was prevalent and the extract of AJJP showed a high cytotoxicity in a metastatic breast cancer cell line.

P 024 ANTIANGIOGENIC PROPERTIES OF TYROSOL AND HYDROXYTYROSOL PRESENT IN FOOD: *EX VIVO* MOUSE AORTIC RING MODEL

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Excessive concentrations of vascular endothelial growth factor (VEGF) trigger angiogenesis, which cause complications such as destabilization of atherosclerotic plaques and increased growth of tumors. Indeed, VEGF and VEGF receptor 2 (VEGFR-2) are molecular targets for drug therapies aiming inhibition of VEGF signaling. Tyrosol (TyrOL) and hydroxytyrosol (HT) bioactives are presents in a limited number of foods such as table olives (0.435–353.5 and 14.5–3833 mg/Kg, respectively), virgin olive oil (VOO) (0.2–32.9 and 0.39–41 mg/Kg, respectively) as well as in wine (1.1–48.3 and 0.00007–9.6 mg/L, respectively). The latter formed by yeast from the metabolism of tyrosine by the Ehrlich pathway during the alcoholic fermentation. The aim of this study was to determine the molecular mechanism underlying the potent inhibition of VEGF signalling by TyrOL and HT bioactives. Experiments included inhibition of VEGF by ELISA assay and their subsequent effect on the downstream signaling pathway (PLCγ1, Akt, and eNOS) by Western blot on human umbilical vein endothelial cells (HUVECs). Additionally, *ex vivo* antiangiogenic effects was examined in a mouse aortic ring model. TyrOL and HT were capable of inhibiting VEGF receptor-2 activation with an IC_{50} value of 38.3 µM and 72.4 µM. TyrOL and HT significantly inhibit PLCγ1 phosphorylation (81% and 41% inhibition, respectively). Furthermore, HT significantly activated eNOS via Akt, while TyrOL maintains eNOS activation. Furthermore, the VEGF-induced microvessel sprouting was significantly inhibited by TyrOL and HT at 96.5 % and 96%, respectively. These data provide new evidence supporting the interest in tyrosol and hydroxytyrosol for their further exploitation as anti-VEGF ingredients in food.

P 026 SILYMARIN CATABOLISM IN AN *IN VITRO* COLON MODEL AND ITS ASSOCIATIONS WITH MICROBIOTA

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Background:

Silymarin (SLM) is a mixture of flavonolignans extracted from the seeds of milk thistle (Silybum marianum). It has demonstrated hepato-protective effects and a range of other activities. SLM is one of the most frequently sold herbal supplements. Studies indicate that phenolic components undergo extensive catabolism in the distal parts of the digestive tract, resulting in the generation of various metabolites. These metabolites can enter the bloodstream and exhibit higher activities compared to their original precursor compounds. Additionally, it is possible that some of the effects exerted by these compounds are influenced by their impact on the gut microbiota.

Objectives:

The main objective was to explore the catabolism of SLM in the colon, examining the range of metabolites produced and their inter-individual variations. Additionally, we aimed to assess the impact of SLM on the composition and metabolic activity of colon microbiota using a multi-omics approach.

Methods:

Using an *in vitro* colon model batch incubations with faeces of 20 individuals we explored the effect of individuals' microbial profiles on the catabolism of SLM (50 µg/mL), and on the other hand, its effect on the microbiota. Illumina 16S rRNA sequencing (NGS), liquid chromatography-mass spectrometry (LC-MS) and 500 MHz nuclear magnetic resonance spectrometry (1H-NMR) were used, and the data from all three platforms were processed separately and by data fusion using PERMANOVA, linear models and block PLS.

Results:

Silymarin, which consisted mainly of silybin B, silybin A, silydianin and isosilybin A as well as four other flavonolignans, was catabolised into a few final products with m/z 469.113, indicating demethylation of its major components. At earlier time points, double bond reduction was observed with subsequent demethylation and both double bond reduction and demethylation in the final products. Silymarin was found to suppress bacterial metabolism, as evidenced by a 2.8% decrease ($p < 0.01$) in the production of short-chain fatty acids (SCFAs), an 8.4% decrease ($p < 0.001$) in branched-chain fatty acids (BCFAs), decreased utilisation of carbohydrates, and amino acids ($p < 0.05$). No effect was observed on the composition of the microbiome itself. Clear associations were found between the abundance of Faecalibacterium, Oscillibacter and Anaerotruncus taxa and increased production of the most abundant early catabolite with m/z 485.144, most probably resulting from early double bond reduction of silybin B and silybin A.

Conclusion:

Using multi-omics approach, we have detected the role of specific bacterial taxa in the colon catabolism of silymarin. Considering the distinct biological effects exhibited by its products, these findings hold significant relevance in the context of personalised nutrition.

Acknowledgment:

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P 028 EFFECT OF ALCOHOLIC FERMENTATION ON THE POLYPHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF A BEVERAGE MADE FROM *Astrocaryum huaimi* MART. (TUCUM DO CERRADO) FRUIT

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Background:

Astrocaryum huaimi Mart. known as Tucum do cerrado is a palm widely found in the state of Goiás (Brazil). This palm tree inhabits in humid places and riverbanks forming dense populations with many fruits. Its low commercial use is due to the lack of studies on its composition and processing technology. A novel project that aims to promote the human consumptiom of Tucum do cerrado, is currently being carried out and several by-products from this fruit such as ice cream, flour, oil and liqueurs have been developed.

Objective:

The objective of this work was to characterise the phenolic composition of Tucum do cerrado fruit by HPLC-HRMS and to evaluate the effect of the alcoholic fermentation process on its composition and antioxidant activity for the first time.

Methods:

Tucum do cerrado samples (peel+pulp) were collected in Goiás-Brazil and transported frozen to the Universidad de Sevilla (Spain). They were then freeze-dried and crushed for the analysis of the starting fruit. The alcoholic fermentation was carried out from lyophilized fruit mixed with water (1:3) which was then centrifuged in order to remove the solid residues (fiber and gums). QA23 yeast strain was used to performed the alcoholic fermentation under controlled temperature (28ºC) and continuous agitation (150 rpm). During the fermentation process, weight, number of yeasts, Brixº, glucose and fructose content and ethanol were measured every 24 hours. Methanolic extracts of the fruit and the fermented beverage were prepared for the analysis of phenolic compounds by HPLC-RHMS and other supplementary measures (total phenols, total carotenes and antioxidant capacity (DPPH and ORAC)).

Results:

Alcoholic fermentation took place over 5 days being glucose and fructose practically consumed from day 2. The final alcoholic strength of the drink was 2%. Twenty-six phenolic compounds were identified in the fruit and nineteen compounds in the alcoholic fermented beverage (mainly flavonoids, hydroxybenzoic and hydroxycinnamic acids) (Figure 1). The alcoholic fermentation process causes the loss of some polyphenols (such as cafeic, ferulic, coumaric and protocatechuic acid) but also the appearance of others that were not present in the original fruit (e.g. gentisic acid, catechol). The amount of total phenols was significantly higher in the alcoholic fermented beverage than in the original fruit (2.84±0.33 µg/mL of galic acid *versus* 2.44±0.37 µg/mL of galic acid). The same trend was observed for total carotenes, which showed a 4-fold increase with respect to the starting fruit. The formation of ethyl alcohol can explain this phenomenon, which is capable of extracting more bioactives from the matrix during fermentation. Regarding the antioxidant capacity, we found very similar IC_{50} values for the fruit and fermented extracts proving that the fermentation not affect the antioxidant activity.

Conclusion:

To conclude, this work has contributed to the in-depth knowledge of the phenolic composition of Tucum do cerrado whose composition is little known. Alcoholic fermentation is a suitable technological process for obtaining a low alcoholic beverage and a high value by-product made from Tucum do cerrado as it is able to protect its bioactives and even increase the amount of carotenes.

P 029 ANTIBACTERIAL ACTIVITY OF SELECTIVELY HALOGENATED FLAVONOLIGNANS

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Flavonolignans are polyphenols typically isolated from milk thistle (*Silybum marianum*). Silymarin (extract from the fruits of *S. marianum*) contains silybin, silychristin, isosilybin, taxifolin, silydianin, and other minor compounds. These compounds and their derivatives generally have low toxicity and interesting biological activity for example hepatoprotective, anti-inflammatory, antioxidant, and modulation of multidrug resistance (MDR) [1]. Selective modifications, such as halogenation, may provide new, previously unknown compounds and increase the biological potential of silymarin flavonolignans.

A library of new halogenated derivatives of flavonolignans (silybins A and B, 2,3-dehydrosilybin, silychristin A, and 2,3-dehydrosilychristin A) was prepared [2,3]. The biological effects of flavonolignan halogenation were investigated. The ability of the halogenated derivatives to inhibit bacterial communication and biofilm formation and to modulate antibiotic resistance in bacteria, as well as their antiradical, reducing, anti-lipoperoxidant, cytotoxic, and anti-inflammatory activities and also their ability to modulate doxorubicin-resistant phenotypes in human ovarian cancer cells were evaluated and compared with their parent compounds.

All halogenated derivatives were able to inhibit the AI-2 type bacterial communication (quorum sensing) at concentrations below 10 µM. The derivatives inhibited the adhesion of bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) to the surface, preventing biofilm formation. These effects suggest that the halogenated derivatives are promising antibacterial agents. Moreover, these derivatives acted synergistically with antibiotics and reduced the viability of antibiotic-resistant *S. aureus*. Some flavonolignans were able to revert the resistant phenotype to a sensitive one, implying that they modulate antibiotic resistance most probably by efflux pump inhibition [3].

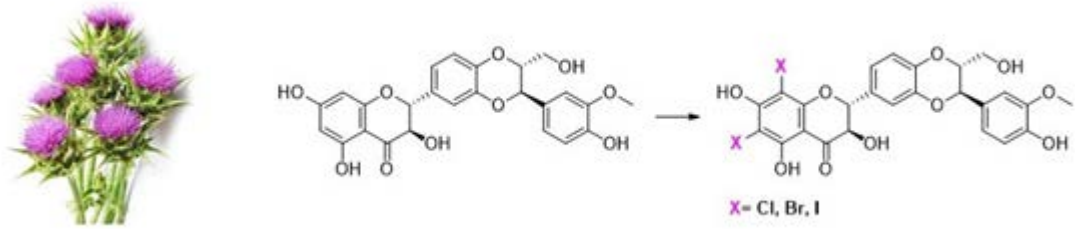

Figure 1: The structure of prepared halogenated flavonolignans.

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P 030 ACCELERATED SOLVENT EXTRACTION AS A GREEN TOOL FOR THE RECOVERY OF LIGNANS FROM SESAME CAKE

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The food industry generates a large amount of waste every year, which opens up a research field aimed at valorizing into high-value products, supporting the concept of zero waste. Sesame cake (SC), a by-product generated from the sesame oil industry, represents a rich source of nutrients and bioactive secondary metabolites, including phenolic compounds. Efficient implementation in food supplements and the nutraceuticals industry could provide a way of this residue valorization, thus achieving the concept of sustainability and circular economy. The main bioactive compounds present in SC are lignans glycosides, which are multi-functional in the human body, presenting several biological activities such as antioxidant, hypolipidemic, hypocholesterolemic, antidiabetic, anticancer, antihypertensive and cardioprotective activities. In addition, "green" extraction techniques that use "green solvents" are gaining wider attention nowadays for extracting bioactive compounds from natural products. This work aims to exploit sesame oil by-products through holistic, environmental-friendly approach. To reach this aim, the performance of accelerated solvent extraction (ASE) as a "green" method for the recovery of lignan-rich extracts from SC to be incorporated into health-promoting food products, was investigated. ASE is a fast and efficient extraction technique that applies a high pressure (1500 psi) for sample extraction in an enclosed system using SST-cells with the additional effect of heat in a controlled oven. The optimal ASE experimental conditions were developed using response surface methodology (RSM). A four-factor Central-Composite Design (CCD), consisting of a total of 27 experimental runs, was used to investigate the effects of four independent variables, X1: ethanol concentration (20–80%), X2: static time (3–7 min of 3 extraction cycles), X3: temperature extraction (65–125 °C) and X4: sample weight (0.5–2 g) on the responses: extraction yield, sesaminol triglucoside (SETRI) and sesaminol diglucoside (SEDI) contents. ASE operated at the condition generated by RSM using a flush volume of 65% and a purging time of 90s. Experimental data were fitted for each response and the model was validated using an analysis of variance (ANOVA) to obtain optimal extraction conditions. The effectiveness of ASE as a "green" extraction technique against the conventional solid-liquid extraction method was compared, in terms of extraction yield and quantity of lignans. Extracts were analyzed by HPLC-DAD-MS for estimation of lignans contents and by ABTS and DPPH assays for evaluation of their antioxidant capacity. SETRI followed by SEDI were the most abundant lignans glucosides in SC extracts. ASE optimal conditions with 83.7% desirability (X1=80%, X2=3 min, X3=65 °C, X4=2 g), resulted in 9.34% extraction yield and 150.34 ± 5.89 mg/g total lignans, in close agreement with the theoretical values predicted by the generated models (9.42%, 151.6 mg/g, respectively). Compared to extracts obtained by the conventional extraction method, those obtained by ASE technology exhibited higher lignan content and antioxidant activity, however, similar results were observed for extraction yield. In conclusion, ASE, as a viable green alternative method, could successfully increase the potential of SC to produce extracts rich in lignans. Moreover, the optimal ASE conditions of this study could serve as a scientific basis for scaling-up industrial production.

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P 031 PHENOLICS AND ANTIOXIDANT CAPACITY OF SAUVIGNON BLANC WINES FROM THE REGION OF DRAMA AS MARKERS OF QUALITY AND TERROIR

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Besides variety, terroir generates unique wines with a composition that differs in phenolic compounds, antioxidant activity, and organoleptic characteristics, revealing the unicity of each region. Drama regional unit is part of East Macedonia and Thrace region in Greece, with more than 510 hectares of vineyards spread over three PGI zones. Over the last decade, vineyards have been extended to other areas of the region, increasing the diversity of the wines produced. Phenolics depend on the grape variety and environmental factors such as soil type, terrain characteristics and climate conditions. The literature reports that terroir affects the phenolic and organoleptic characteristics of wines. Phenolics in wine influence the taste and can be markers of wine quality and authenticity.

However, the phenolic content and total antioxidant capacity of Sauvignon blanc wines from the winemaking areas of Drama in Greece remain unclear. Thus, this work aimed to compare levels of phenolics, flavonoids, antioxidant capacity, and organoleptic characteristics of Sauvignon blanc wines in eight sub-zones of PGI Drama.

The wines were produced on a laboratory batch-scale (25 kg each) using the same procedure to avoid variations from the winemaking process. Only mature, intact grapes of the Sauvignon blanc variety were used. After the hand removal of stems, the grape berries were hand crushed to obtain the juice. The grape juice was inoculated with 25 g/hL of *Saccharomyces cerevisiae*, and the fermentation was done in duplicate under controlled temperature conditions in 30 L stainless steel vats. The total phenolic (TPC) and flavonoid content (TFC) were determined with Folin–Ciocalteu, and the aluminum complexation method, respectively. The antioxidant capacity was measured by three different analytical assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) and ferric ion reducing antioxidant power (FRAP). The wines have been subject to a sensory evaluation by ten trained panelists.

The white wines studied were high in phenolics and antioxidant activity, although not higher than red wines, offering the possibility of raising awareness about their value in a nutritional sense. Wines produced showed differences in the amount of phenolics, flavonoids, and their antioxidant capacity, suggesting that terroir plays a primary role in this variation. Moreover, sensory analysis confirms differences in the sensorial parameters among the wines. Not all the sensory attributes were associated with phenolic and flavonoid content and antioxidant capacity. Correlation analysis revealed that aroma intensity was positively related to the altitude (0.716, p=0.046). On the other hand, TPC and DPPH negatively correlate with tannins, whereas ABTS correlates positively with the aftertaste attribute. FRAP antioxidant capacity of the wines was positively correlated with taste quality and fruity taste parameters and negatively with acidity. Overall, the phenolics and antioxidant capacity of wines vary depending on the terroir but seems that their amount relates to the final taste and texture.

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P 032 THE IMPACT OF COFFEE-DERIVED CHLOROGENIC ACID ON COGNITION – A SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction:

Caffeinated and decaffeinated coffee consumption has been associated with some beneficial effects on cognitive function. These effects have been associated with the polyphenol family of chlorogenic acids (CGA).

Objectives:

The aim of this review was to evaluate and synthesise evidence of the relationship between coffee-derived CGA and cognitive function.

Methods:

Various databases were searched and references of included studies were hand-searched, yielding 6 randomized control trials (RCTs) and 17 longitudinal studies. Studies examining the effect of coffee-derived CGA, stating dosages used, on cognitive function in human subjects using standardised measures of cognition were included. Studies without control groups or placebos were excluded except for longitudinal studies. A descriptive synthesis and two subgroup meta-analyses were conducted with the RCTs to explore the overall effect of CGA on cognitive function by study and by cognitive domain.

Results:

The systematic review of RCTs indicated some benefits of coffee CGA for executive function, attention, motor activity, and mood when consuming 300–1106 mg CGA. However, the overall meta-analysis did not show a significant effect of CGA on cognition. The evidence from prospective cohort studies indicated that moderate coffee drinking (3–5 cups daily) was associated with a lower risk of dementia and Alzheimer's, while low coffee consumption (0–2 cups daily) was associated with greater risk of cognitive impairment.

Conclusion:

Based on the findings from the narrative systematic review, there is some evidence that consumption of CGA from coffee may improve executive function, attention, motor activity, and mood and is associated with a lower risk of developing dementia and Alzheimer's. However, the non-significant meta-analysis indicates that the evidence base from good quality RCTs is limited. Further research is required to explore cognitive effects of CGA from coffee.

P 033 GLUCURONIDATION OF PHENOLIC ACIDS

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Glucuronidation represents a key metabolic pathway for various (poly)phenols (e.g. flavonoids); as such, glucuronides of polyphenols are of great interest in metabolic studies. In organism, bioconjugation of a polyphenol with a glucuronyl moiety is catalyzed by UDP-glucuronosyltransferases. However, a similar enzymatic approach is not always feasible for synthesis due to a variety of factors (high cost of reactants, low stability etc.).

Phenolic acids are known metabolites of flavonoids; however, their glucuronides have mostly been ignored in terms of both synthesis and their biological properties. We synthesized several glucuronidated phenolic acids (Fig. 1), which will be used in the study of metabolism of flavonoids.

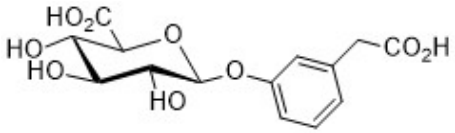

Figure 1: Example of synthesized glucuronide.

This work was supported by the Czech Science Foundation (23-04654S).

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P 034 BIOMARKER COMPOUND DETERMINATION USING MODERN INSTRUMENTAL METHODS AS A TOOL FOR HONEY FLORAL ORIGINS EVALUATION

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Honey is a well-regarded food product with antibacterial properties and other health benefits, which is pre-eminent compared to the majority of other sweeteners. The rivalry between honey manufacturers is huge and there is a need for a way to stand out. Monofloral honey is a product, which is relatively hard to obtain since bees have to gather the majority of honey from a single type of species, but when is produced, the product often has unique organoleptic, taste and color properties. Compared to polyfloral honeys, monofloral accumulates more specific compounds from the floral source. The polyphenol, volatile organic compound (VOC), macro- and trace element profiles are considered prominent characteristics for monofloral honey evaluation. The aim of the study was to develop a what-to-do strategy for beekeepers to confirm their honey floral origins using modern analysis methods.

The ultra high performance liquid chromatography- high resolution mass spectrometry (UPLC-HRMS), gas chromatography- mass spectrometry (GC-MS) and inductively coupled plasma- mass spectrometry (ICP-MS) methods were chosen modern instrumental methods to evaluate mentioned physicochemical profiles. The nuclear magnetic resonance (NMR), Fourier transformation infrared spectroscopy (FT IR) patterns and fingerprint stable isotope ratio values obtained by isotope ratio mass spectrometry (IRMS) were performed as well for monofloral honey chemical evaluation. Seven in Eastern Europe common monofloral types were investigated- buckwheat, clover, heather, linden, rapeseed and willow. 40 monofloral and 56 polyfloral honey samples with melissopalynology examinations were used in this study.

Results of the phenolic profile show prominent use of abscisic acid, *p*-hydroxybenzoic acid, p-coumaric acid, rutin and pantothenic acid as biomarker compounds for floral evaluation while using chemometric methods the chemical patterns suggest distinguishing between buckwheat, heather and linden honey. The preliminary VOC profiles show phenylacetic acid derivatives, furfural and isobutyric acid as potential VOC biomarker compounds. Macro- and trace element profiles show characteristic concentrations of elements in honey of Latvian origins and can be successfully used for floral origins evaluation because of different plant bioremediation abilities. For example, heather honey show increased concentrations of K, Ca, Mn, Fe, As, Rb, Cs, Ba, Tl. The NMR patterns show the most versatile discrimination between monofloral groups but in the current studies, it is hard to use for quantification purposes. The FT IR was not successful for the floral source determination but it is still considered a valuable instrumental method because of its ability to determine adulterants and will be included in the proposed what-to-do strategy. Achieved results further will be implemented further for “medical grade” honey preposition to wound healing bandages.

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P 035 INTERACTIONS OF 7,8-DIHYDROXYFLAVONE WITH BIOGENIC METALS

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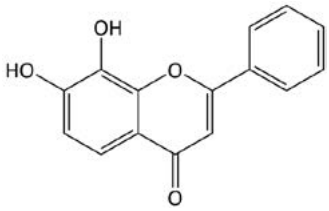
Background and objectives:

7,8-dihydroxyflavone (78DHF), also known as tropoflavin, is a naturally occurring flavonoid found in several plants that are used in traditional medicine. In the last decade, it has been very largely investigated due to its possible health-benefits. However, the interactions of 78DHF with trace metals have not yet been systematically tested. Therefore the aim of this work was to determine these interactions (chelation and reduction) and their consequences (ability to impact metal-triggered Fenton reaction and red blood cell lysis).



Methods:

Firstly, the degree of copper, iron, zinc and cobalt chelation and Cu^{2+}/Fe^{3+} reduction was established by use of *in vitro* spectrophotometric methodologies at four patho/physiologically relevant pH values (pH 4.5, 5.5, 6.8 and 7.5). Then, hydroxyl radical production *via* the Fenton reaction was monitored by an *in vitro* HPLC method coupled with coulometric detection. In the last step, the *ex vivo* copper-triggered lysis of human erythrocytes was tested.

Results:

78DHF did not chelate Cu^{2+} and Cu^{+} ions significantly and was only slightly effective in the case of Zn^{2+} and Co^{2+} ions. In contrast, it showed a good chelating activity toward Fe^{2+} and Fe^{3+} ions. Simultaneously, it was able to reduce both Cu^{2+} and Fe^{3+} ions. It potentiated the iron-catalyzed Fenton reaction at neutral and slightly acidic pH values while inhibition was observed under acidic conditions. The effect on the copper-catalyzed Fenton reaction was again antioxidant at pH 4.5 whereas significantly pro-oxidative only at pH 6.8. The impact on red blood cell lysis did not correspond to the Fenton experiments. 78DHF protected red blood cells from copper toxicity.

Conclusion:

78DHF was not an effective chelator of biogenic metals with exception of iron ions. However, it protected human red blood cells from copper-induced lysis in *ex vivo* conditions.

P 036 MONITORIZATION THE PHENOLIC PROFILE OF A FRESHLY EXTRACTED OLIVE OIL AFTER DECANTING FOR 3 MONTHS

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Olive oil, a basic ingredient in the Mediterranean diet, is of great interest today due to its health benefits. Its consumption is associated with the prevention of degenerative diseases and a low incidence of cardiovascular pathologies and different types of cancer, among others illnesses. Part of its beneficial properties are due to its unique phenolic composition, notably its contents in tyrosol, hydroxytyrosol and oleuropein, among other polyphenols. The quantitative and qualitative phenolic content of olive oil is widely influenced by many variables related to the production process, from the state of ripeness of the olive fruit to the storage and conservation conditions. For this reason, one of the fundamental steps for obtaining this healthy oil is decanting, a natural process by which, thanks to the difference in density of the components, the olive oil is separated from the water and the suspended solids still present.

The aim of this research was to characterize the phenolic composition (qualitative and quantitative) of a freshly extracted oil, in order to monitored its evolution during a 3-month decanting period by advanced analytical techniques. The extraction of phenolic compounds was carried out by an optimized liquid-liquid extraction using hydro-methanolic mixtures. Briefly, 5 grams of oil were weighed and homogenised with n-hexane. For the extraction of phenolic compounds, a mixture of MeOH:H_2O was used to liquid-liquid extraction (80:20, v:v) . After centrifugation, the hydromethanolic phase was separated from the oil residue. This step was repeated twice. Finally, the obtained extracts were dried using a vacuum rotary evaporator, and subsequently the extracts were reconstituted in methanol and filtered for their analysis.

Thanks to this study, it was possible to compare the phenolic content of the oil samples, freshly extracted and after decanting for 3 months, thus showing the influence of the decanting process on the analytes of interest. The use of optimized extraction and analytical procedures allow to put into light the evolution that suffers the phenolic compounds in this important technological process.

P 037 EXPLORING THE POTENTIAL OF COFFEE PULP IN REGULATING HYPOLIPIDEMIC PROPERTIES AFTER *IN VITRO* DIGESTION

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The coffee pulp is the main by-product obtained during the wet processing of coffee, which reveals high content of phenolic compounds and caffeine. The aim of this study was to evaluate the effect of the digestion process on the biological potential of of coffee pulp extract (CPE) and flour (CPF) to prevent NAFLD in the HepG2 cell line after induction of intracellular fat accumulation. Our results show that the CPF and CPE contain a high amount of phenolic acids (chlorogenic and gallic acids), as well as flavonoids (quercetin derivatives). Caffeine exhibits higher concentrations than phenolic compounds in CPF and CPE. The hepatocyte exposure to palmitic acid (PA) caused an increase in ROS production (2.4-fold, $p<0.05$) that was reversed when cells were treated with the CPF and CPE digested fractions of the different digestion phases. The results brought out that the digested fractions of CFP and CPE and their raw materials were able to significantly reduce the increase of ROS, highlighting the intestinal phase from CPE and CPF which significantly ($p<0.05$) reduce the increase of ROS in 63% and 36%, respectively. Lipid accumulation in the cell line was qualitatively assessed by lipid staining. An increase of intracellular lipids was observed in PA-treated hepatocytes (1.3-fold, $p<0.05$) comparing to non-treated cells, being reduced when cells were cotreated with CPF and CPE intestinal phase digested fractions and CPE raw material (78, 121, 82%, $p<0.05$, respectively), highlighting the capacity to reverse lipid accumulation exhibited by intestinal phase in both cases (CPF and CPE). The release of phenolic compounds in these matrices during digestion process were inversely correlated with intracellular lipid accumulation. A similar behavior was observed for accumulation of intracellular tryglicerides (TAGs), all cotreatments significantly reduced (43–101%, $p<0.05$) the increase in TAG caused by PA treatment. In addition, PA stimulation significantly reduced (35%, $p<0.05$) lipase activity compared to non-treated cells. The effect found during the digestion process of these ingredients in the lipolysis may be probably related to the release of phenolic compounds and caffeine, since chlorogenic acid, 3,5-dicaffeoylquinic acid, quercetin 3,7-dihexoside, p-coumaric acid, caffeine, were highly correlated with the lipase activity. Therefore, these compounds present during the CPF and CPE digestion process could promote fatty acid oxidation.

Results from the biological activity of CPF and CPE after *in vitro* static digestion in HepG2 cell lines indicate the digested intestinal phases were the main phases fractions capable of regulating lipid metabolism and buffered the adverse effects derived from PA. Therefore, since their hypolipidemic properties have not been modified during the digestion process, CPF and CPE could act as modulators of all the pathways involved in the accumulation of lipids in the liver and could be a key element in the prevention of hepatic lipid accumulation and eventually NAFLD.

P 038 METABOLIC PROFILING OF HEALTH BENEFICIAL PHYTOCHEMICALS IN VERTICALLY-FARMED AND GLASSHOUSE-GROWN BASIL

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Controlled Environment Agriculture (CEA), often termed "Vertical Farming", is an emerging technology that can influence UK food security and resilience by growing often imported foods closer to consumer communities. Shortening supply chains and reducing reliance on imports may be important in a global food supply system already buffeted by Covid, Brexit, trade changes, and which must adapt to the uncertainties of Climate Change. The complete controllability of growth conditions under CEA gives potential to stretch crop phenotype to manipulate crucial commercial factors such as yield, time to harvest, and desired plant habit (e.g., leaf / stem ratio) but also to manipulate the biochemical pathways that contribute to flavour, nutritional value, health benefits, and ultimately consumer experience.

In this collaboration with Intelligent Growth Systems, we examined effects of their total CEA (TCEA) system on the quality of basil as a model plant. Basil grown under TCEA conditions was substantially quicker to harvest than glasshouse-grown material and was not discernibly different in apparent consumer quality. In addition, metabolic profiling showed that glasshouse- and TCEA-grown basil had generally similar phytochemical profiles with high levels of characteristic antioxidant polyphenols 1, such as rosmarinic acid and chicoric acid. Generally, variation in phytochemicals noted between the growing methods was no greater than within replicate sub-samples of the TCEA and glasshouse-grown material. Studies with different light regimes in TCEA that altered plant habit are in progress to discern if these can induce corresponding changes in the levels of these potentially health beneficial components.

Vertically-farmed Basil

P 039 THE EFFECT OF COOKING METHODS ON THE TOTAL MONOMERIC ANTHOCYANIN CONTENT IN BLACK RICE BY THE pH DIFFERENTIAL METHOD

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Background:

Black rice (*Oryza sativa* Linn.) contains high amounts of anthocyanins mainly as cyanidin 3- glucoside and peonidin 3-glucoside. Previous studies report various health benefits of black rice both *in vitro* and *in vivo* including anti-inflammation, antioxidant, anti-diabetes, and neuroprotection. Even though black rice is a good source of anthocyanins, it shows a significant loss of anthocyanins after the cooking process.

Objectives:

This study aims to evaluate the effect of five common cooking methods (boiling, steaming, microwave, rice cooker and risotto) on the anthocyanin content of black rice.

Method:

The 60% and 100% of methanol acidified with 1.0 N HCl (85:15, v/v) were applied to extract the three black rice varieties: Riceberry rice (RR), and Nerone black rice (NR) and Giant wild rice (GR) for assessing the extract conditions. The different common cooking methods were applied in RR and NR. The total monomeric anthocyanin content was measured by the pH differential method with results expressed as cyanidin-3-glucoside.

Results:

The anthocyanin contents in raw RR, NR and GR were 59.91, 219.03 and 2.26 mg/100 g dry weight by using 100% acidified methanol extraction. The 60% of acidified methanol extraction gave lower anthocyanin contents; 41.04, 180.34 and 1.55 mg/100 g dry weight, respectively. The different cooking methods had significant loss of anthocyanins in RR, and NR, approximately 41–57%. However, there were no significant differences between cooking methods in both black rice varieties.

Conclusion:

Our findings demonstrate that thermal treatment affects the stability of anthocyanins in black rice. A preventive approach needs to be applied to prevent thermal degradation during the cooking process. In addition, the mechanism of thermal anthocyanin degradation and their derivatives need to be explored.

P 040 INCREASING THE BIOAVAILABILITY OF FLAVONOIDS USING DOUBLE-COATED NANOLIPOSOMES

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[2] *Wageningen University and Research, Food and Biobased Research, Wageningen, Netherlands*

Background:

Encapsulation is commonly used to protect bioactive compounds in food from degradation. Spray drying is widely used for heat-labile nutraceuticals, and maltodextrin (MD) has been employed for encapsulation of polyphenols like tea polyphenols and curcuminoids. Freeze drying is suitable for heat-sensitive bioactives. Cyclodextrin(CD) is favored for encapsulating hydrophobic compounds like flavanones because of its hydrophobic cavity. Nanoliposomes (NL) have emerged as promising drug delivery systems, although their vulnerability to gastric and intestinal fluid breakdown remains a challenge. Several polysaccharides have shown the promising protective effect when they are coating on the surface of liposomes by electrostatic interaction, such as chitosan and pectin, sodium alginate and chitosan. Flavanones are the aglycons of glycosides that often appear in plants. They are regarded as the bioactives and offer health benefits. However, their poor water solubility limits bioavailability, which is a common issue for hydrophobic substances. Hesperetin (HST), a flavanone, exhibits antioxidative and anti-inflammatory properties, but the poor water solubility of hesperetin limits its bioavailability to some extent. Overcoming these solubility challenges and enhancing the delivery systems for flavanones could unlock their full potential in promoting human health.

Objective:

To increase the bioavailability of flavones by comparing different delivery systems.

Methods:

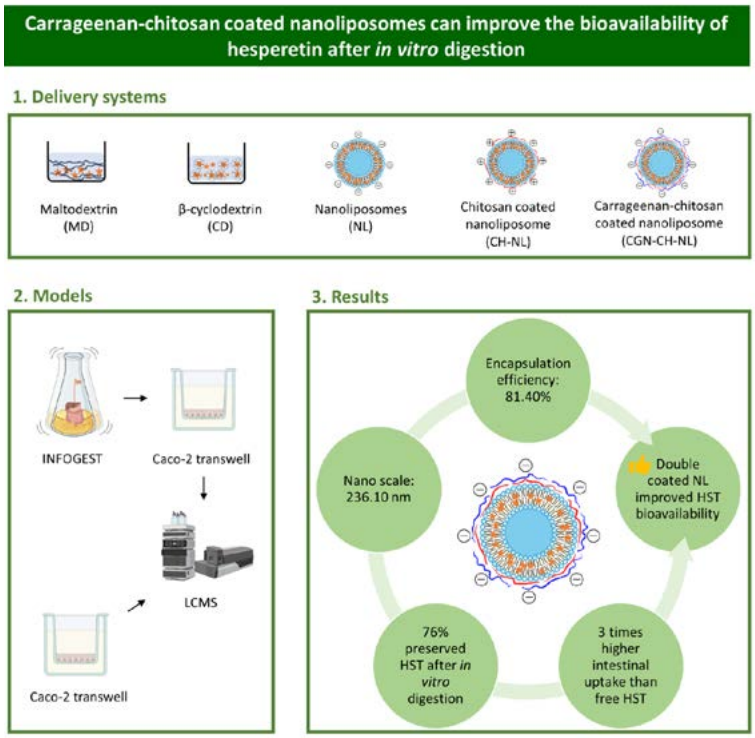
HST was used as a model compound for flavanones. HST was encapsulated in MD and CD by spray-drying and freeze drying, respectively. NL were made by the thin film hydration method followed by sonication. The coated NL were prepared by drop-wise chitosan (CH) and carrageenan (CGN) solutions. Size and Zeta potential, the encapsulation efficiency (EE) and the loading capacity (LC), were characterized. Thereafter, a release profile during *in vitro* digestion was determined for all delivery systems and cellular uptake and transport were investigated both before and after digestion. Finally, phenolic metabolites were defined.

Results:

Physical characterization showed circle equivalent diameter of MD and CD were 8.63 ± 4.42 µm and 22.84 ± 24.55 µm, respectively. The NL and coated NL were between 145.90–236.10 nm in size with even distribution. NL had the highest EE (81.40%), whereas CD had the highest LC (56.38%). Undigested CD allowed the highest transport of HST over the Caco-2 barrier during 6 hour incubation. However, after *in vitro* digestion, 76% of delivered HST was effectively kept in the CGN-CH-NL, whereas delivery systems such as MD and CD retained in the carriers only the 30% and 66%, respectively. CGN-CH-NL showed the highest transported HST through the intestinal epithelium and maintained its transport at 9 ng after 6 hours exhibiting a threefold increase compared to free HST. PCA analysis of phenolic metabolites reveals changes in cellular metabolism.

Conclusion:

The CGN-CH-NL delivery system prevents the release of HST during *in vitro* digestion and improves cellular uptake. Polysaccharide coated nanoliposomes can be an interesting tool for the delivery of flavonoids or drugs to the small intestine and improve their transepithelial transport.


Carrageenan-chitosan coated nanoliposomes can improve the bioavailability of hesperetin after *in vitro* digestion
1. Delivery systems
Maltodextrin (MD)
β-cyclodextrin (CD)
Nanoliposomes (NL)
Chitosan coated nanoliposome (CH-NL)
Carrageenan-chitosan coated nanoliposome (CGN-CH-NL)
2. Models
INFOGEST
Caco-2 transwell
LCMS
Caco-2 transwell
3. Results
Encapsulation efficiency: 81.40%
Double coated NL improved HST bioavailability
3 times higher intestinal uptake than free HST
76% preserved HST after *in vitro* digestion
Nano scale: 236.10 nm

P 041 IDENTIFICATION OF AGGREGATE PHENOLIC METABOTYPES AFTER AN ORAL (POLY)PHENOL CHALLENGE TEST (OPCT) AND THEIR ASSOCIATION TO THE CARDIOMETABOLIC HEALTH STATUS OF 300 SUBJECTS

Cristiana Mignogna[1], José Fernando Rinaldi de Alvarenga[1], Claudia Favari[1], Nicola Bragazzi[1], Vicente Agulló[1], Elisa Monica[1], Maria Sole Morandini[1], Alice Rosi[1], Nicole Tosi[1], Valentina Spigoni[2], Raffaella Aldigeri[2], Annalisa Bianchera[1], Letizia Bresciani[1], Giovanni Frascio[2], Giulia Frigeri[2], Silvia Seveso[2], Roberta Musa[3], Paola Cerati[3], Rosalia Aloe[3], Francesca Turroni[4,5], Valeria Barili[2], Davide Martorana[6], Francesca Scazzina[1], Ruggero Bettini[1], Marco Ventura[4,5], Alessandra Dei Cas[2], Riccardo Bonadonna[2], Furio Brighenti[1], Daniele Del Rio[1,5], Pedro Mena[1,5]

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4 *University of Parma, Department of Chemistry, Life Sciences, and Environmental Sustainability, Parma, Italy*
5 *University of Parma, Microbiome Research Hub, Parma, Italy*
6 *University Hospital of Parma, Medical Genetics, Parma, Italy*

Background:

Increasing evidence shows that (poly)phenol-rich diets are associated to a reduction of non-communicable diseases. However, heterogeneity in the bioavailability and physiological response to the consumption of these food bioactives can affect their efficacy. Individuals showing similar metabolic profiles for specific (poly)phenols can be clustered into phenolic metabotypes.

Objectives:

This study aims at identifying aggregate phenolic metabotypes related to a wide range of dietary (poly)phenols, and at assessing the cardiometabolic health status of the individuals belonging to each metabotype.

Methods:

An intervention study was carried out on 300 healthy volunteers (18–74 y) to collect dietary and lifestyle information, clinical data and biological samples. Subjects underwent a standardised oral (poly)phenol challenge test (OPCT) consisting in an acute supplementation of up to 15 classes of dietary (poly)phenols. Urine samples collected during the following 24-h were analysed through UPLC-IMS-HRMS to assess the individual urinary excretion of phenolic metabolites, allowing clustering according to aggregate metabotypes. Blood samples were analysed to determine common cardiometabolic health biomarkers and for whole-genome genotyping. Faeces were subjected to microbial profiling to determine gut microbiota composition at species level. Cardiometabolic risk scores were also assessed.

Results:

Up to 298 volunteers finished the study. Preliminary results on 180 subjects (51.3% women, 36.1 y (SD ± 14.9)) indicated that 72% of the sample had a normal weight, 23% was overweight and 5% obese. A preliminary targeted approach was performed for the identification of about 130 (poly)phenol metabolites and population clustering according to different metabotypes. The preliminary results on 180 subjects out of 298 showed two main metabotypes defined by differences related mainly to the gut microbiota composition. Final data on metabotypes and cardiometabolic risk are presented in the poster.

Conclusions:

Individuals metabolise dietary (poly)phenols in different ways and the interlink among different families of (poly)phenols have been described. Further analyses are ongoing to provide a deeper knowledge on inter-individual variability determinants involved in metabotype formation and its relation to the cardiometabolic health status.

Funding Sources:

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (PREDICT-CARE study, Grant Agreement N. 950050).

P 042 PRELIMINARY CHARACTERISTIC OF THE FIRST BULGARIAN PLUM-APRICOT HYBRID "STENDESTO"

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The high commercial value of Prunus fruits leads to the demand for new cultivars and interspecific hybrids. The only successful Bulgarian plum-apricot hybrid "Stendesto" is currently poorly studied. This variety could comprise potential benefits based on information for its parents: the "Stanley" plum and the "Modesto" apricot. The present study aimed at providing a preliminary characterization of the biological potential of "Stendesto" fruits in terms of phenolic compounds, considering both free and bound phenolics. Furthermore, the *in vitro* antioxidant potential of the extracts was revealed, with an additional focus on alpha-glucosidase inhibitory activity.

A higher content of total phenolics was found in the free phenolic fraction 23.52±0.75 mg GAE/g dw compared to the two studied bound phenolic fractions - 0.72±0.008 and 0.39±0.005 mg GAE/g dw, respectively. They contained more than 30 times less total phenolics contrasted to those obtained by alkaline extraction. The same trend was followed by the results regarding the total flavonoids, total monomeric anthocyanins, and antioxidant activity. The highest content and activity were respectively found for the free fraction, and when considering the bound fraction, the results were remarkably lower. However, when comparing the two extraction approaches of the bound phenolic compounds, alkaline hydrolysis resulted in a better yield and was thus revealed to be more efficient.

The alpha-glucosidase inhibitory potential was also tracked showing the potential to inhibit the enzyme by free phenolic and bound alkaline hydrolysis extracts.

In summary, the preliminary conducted characterization revealed the ‚Stendesto' plum-apricot hybrid as worthy of attention and with potential for further investigation of its nutritional value, phytochemical composition, and biological activity.

Acknowledgment:

This work was supported by the Bulgarian National Science Fund, project no. КП-06-Н67/2 (granted to Aneta Popova). The authors would like to acknowledge prof. Argir Zhivondov for actively working on expanding plumcot varieties in Bulgaria, and registering the "Stendesto".

P 043 ALPHA-AMYLASE INHIBITION AND ANTIOXIDANT EFFECT OF REGULAR AND DECAFFEINATED COFFEE CAPSULE BEVERAGES IN CACO-2 CELLS

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Coffee is the second most consumed beverage in the world after water and is rich in phenolic compounds with antioxidant properties, such as chlorogenic acids, with clinical studies suggesting that higher coffee consumption may be associated with a lower risk of mortality from cardiovascular disease and diabetes.

The aim of this study was to examine alpha-amylase inhibition and the antioxidant effect of espresso coffee capsules.

Two types of commercial espresso capsules (one traditional and one decaffeinated) were purchased from grocery stores in the city of São Paulo, Brazil. The beverages were prepared as described on the package using a household coffee maker. *In vitro,* digestion was performed by hydrolysis with digestive enzymes by Infogest protocol. Alpha-amylase analysis was performed on the digestion samples from the oral and intestinal phases. Caco-2 cells were seeded and differentiated in plates. A solution containing different concentrations (500, 750, 1000, and 2500 µg/mL) of coffee beverages was added to plates. The catalase enzyme activity was measured using the Catalase (CAT) Activity Assay and the antioxidant activity was also evaluated by the Oxygen Radical Absorbance Capacity (ORAC) assay.

Regarding the ability to inhibit alpha-amylase, in the oral phase, regular coffee showed IC50 0.020 mg9 mLR^2=1) and decaffeinated coffee IC50 0.075 mg mL (R^2=0.92). In the intestinal phase, regular coffee showed an IC50 of 0.0098 mg mL (R^2=1), and decaffeinated coffee showed an IC50 of 0.003 mg mL (R^2=0.90).

The antioxidant enzyme activity (Catalase) showed results ranging from 134.12 ≠ 0.75 to 184.42 ≠ 7.32 mg protein /mL in the basolateral portion at 2 h, showing no statistical difference ($p=<0.05$) when compared with regular coffee and decaffeinated coffee in different concentrations. However, when verified the activity of the catalase enzyme in the apical portion, during 2 h in the Caco-2 cell, a statistical difference was observed (Tukey test), in which the drink of regular coffee in the concentration of 500 µg/mL showed better activity of catalase (251.20 ≠ 0.59 mg protein /mL) when compared with the decaffeinated drink and its concentrations. Results well matched the values obtained in other studies with similar cell models. The antioxidant activity of regular and decaffeinated coffee in contact with the Caco-2 cell by the ORAC method ranged from 18.32 ≠ 1.97 to 48.87 ≠ 0.82 µmolar Trolox equivalent / 60 mL drink, showing better antioxidant activity in the decaffeinated coffee drink (48.87 µmolar Trolox equivalent / 60 mL drink) when compared to regular coffee.

Both regular and decaffeinated coffee showed good antioxidant activity. Decaffeinated coffee showed better antioxidant activity in ORAC, and regular coffee showed better enzymatic activity in catalase. In both the oral and intestinal phases, the alpha-amylase enzyme showed good inhibition.

P 044 CAN FLAVONOIDS CHELATE COBALT IONS?

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Introduction:

Rare cobalt intoxications following its release from a metal-based prosthesis are generally clinically severe. There are currently no approved drugs for cobalt intoxication, and therefore there is a need to develop a selective cobalt chelator.

Methods:

As flavonoids are known for their metal chelating properties and safety, a total of 23 flavonoids were screened for their chelating properties using our recently developed novel spectrophotometric assay. In addition, positive or negative consequences of cobalt chelation were tested both *in vitro* and *ex vivo*.

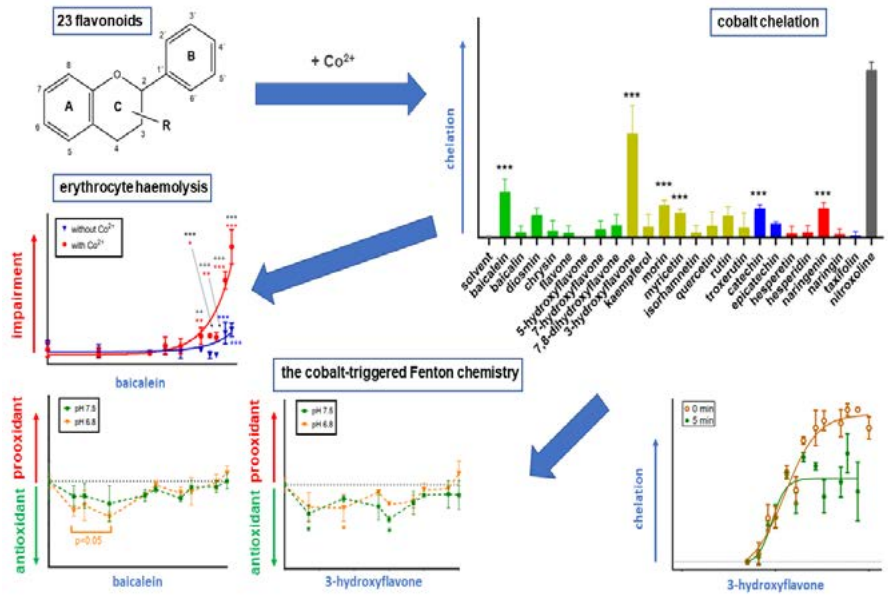
Results:

Six flavonoids chelated significantly cobalt ions at pH 7.5 whereas 13 even at pH 6.8. Solely baicalein demonstrated a significant activity at pH 5.5. However, none of the tested flavonoids was active even at pH 4.5. In general, mentioned baicalein and 3hydroxyflavone were the most active. Both compounds also mildly decreased the cobalt-triggered Fenton reaction. Conversely, baicalein toxicity toward red blood cells was strongly stimulated by the addition of cobalt. Quercetin, an example of flavonoid unable to significantly chelate cobalt ions, stimulated both the cobalt-based Fenton reaction and erythrocyte lysis in the presence of cobalt.

Conclusion:

3-Hydroxyflavone may serve as a potential template for the development of novel cobalt chelators.

Graphical abstract:



P 045 CHRONIC EFFECT OF A PROPRIETARY BOTANICAL BLEND RICH IN (POLY)PHENOLS ON FLOW-MEDIATED DILATION IN AN AT RISK GROUP: A RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED, INTERVENTION STUDY

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Endothelial dysfunction is characterized by impaired endothelium-dependent dilation and is an early predictor of various cardiovascular events in at-risk populations[1]. (Poly)phenols are naturally occurring bioactive compounds derived from various plant species and obtained through the consumption of fruits and vegetables[2,3]. (Poly)phenols are associated with improvement of endothelial function and may protect against cardiovascular and degenerative eye diseases, through effects on the macro and microvasculature[4,5]. Such effects may be mediated by microbiota-derived metabolites i.e. phenyl-γ-valerolactones (PVL's) and phenylvaleric acids (PVA's) rather than the *in planta* parent compounds which have limited bio-availability[6].

Consequently, a double-blind, randomized, placebo-controlled parallel study will be conducted in 62 healthy adults (18–60 years) with at least one risk factor of suboptimal endothelial function; Overweight (BMI ≥ 25 kg/m^2 and ≤ 30 kg/m^2) or waist circumference ≥ 80 cm for women and ≥ 94 cm for men. Participants will consume either a proprietary (poly)phenol-rich extract (≥600 mg) or a maltodextrin placebo for a four-week period. During the study, clinic visits will occur on three separate occasions, weeks 1, 4 & 5 (1-week washout post-intervention). Efficacy of the (poly)phenol extract will be assessed through ultrasound (UNEX EF38G) measurement of Endothelium-Dependent Flow-Mediated Dilation (ED-FMD) of the brachial artery in response to induced ischemia (200 mmHg for 5 minutes) and expressed as FMD-response and relative change (%). The effect on blood pressure will also be measured. Optical Coherence Tomography (OCT) will be assessed, in participants at resting state, using the Heidelberg OCT to measure changes in microvascular diameter in retinal blood vessels and choroidal thickness will be determined. Blood samples will be collected at each visit and plasmatic PVL's and PVA's and parent (poly)phenols will be quantified by MSn[6]. In addition, nitric oxide (NO) metabolites and endothelin-1 will also be quantified and an ACE inhibition assay conducted to explore potential mechanisms of action for the associated vascular improvements.

The results obtained from this study will provide valuable insight to the protective effects of this proprietary (poly)phenol rich extract on macro and microvascular function and their mechanisms of action supporting its use as a therapeutic agent offering protection for vascular and ocular health.

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P 047 PHYTOCHEMICALS PRESENT IN A NEW ZEALAND APPLE CULTIVAR ARE BIOAVAILABLE IN HUMANS, INCREASE BLOOD ANTIOXIDANT RESPONSE AND INHIBIT GROWTH OF LUNG AND BREAST CANCER CELLS *IN VITRO*

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Background:

Apple consumption is associated with improved health and reduced risk of cancer which is attributed to their phytochemical content. Evidence suggests that apple phytochemicals affect different hallmarks of cancer and reduce oxidative stress, which is involved in the pathology of cancer. The limiting factor to obtain these effects in the human body is apple phytochemicals' low bioavailability. The plasma phytochemical metabolites after apple consumption and their effects on plasma antioxidant capacity and cancer cell growth have not been extensively studied.

Objectives:

Our study is focused on a heritage apple cultivar discovered in New Zealand- Monty's Surprise. Based on our previous LC-MS analysis this apple contains high phytochemical (mainly flavonoids) concentrations in comparison with commercial apple cultivars.

This study aims to evaluate the bioavailability of Monty's Surprise apple phytochemicals in humans and their effects on blood total antioxidant capacity and lung and breast cancer cells proliferation.

Methods:

Twelve healthy participants received either apple puree or a placebo as a control in a randomized crossover human study. Blood samples were collected after overnight fasting and at regular intervals up to eight hours post-meal consumption. The main phytochemical metabolites in the participant's plasma were evaluated by LC-MS-MS. Plasma total antioxidant capacity was evaluated by Ferric Reducing Antioxidant Power. Based on the results from metabolomics analysis we created a synthetic mixture of these metabolites and evaluated their effects on viability of lung and breast cancer cells *in vitro* using SYBR green assay.

Results:

The analysis of the Monty's Surprise apple phytochemical metabolites showed that some of the flavonoids and phenolic acids metabolites are present in the blood one hour after the consumption of the apple puree. Moreover, Monty's Surprise apple puree consumption significantly increased ($p < 0.001$) plasma total antioxidant capacity 30 minutes post-meal intake (from the baseline to 170.76 µmol/L ± 34.58), when compared to the placebo consumption. In addition, a synthetic mixture of the Monty›s Surprise apple phytochemical metabolites inhibited lung and breast cancer cell proliferation.

Conclusion:

Results from this study demonstrated that some of the Monty's Surprise apple phenolic compounds are absorbed and enter the systemic circulation after apple puree ingestion and their absorption improves plasma antioxidant status. Moreover, Monty's Surprise apple blood metabolites inhibit growth of lung and breast cancer cells *in vitro*. These findings suggest that incorporating Monty's Surprise apple into the diet may improve human health and prevent cancer development.

P 048 PHENOLIC COMPOSITION OF SNACKS BASED ON PEA PROTEIN, PEA STARCH AND AN OAT B-GLUCAN RICH FRACTION

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The combination of cereals and pulses may play an important role to provide food with a balanced amino acid composition and enough micronutrients. Extrusion technology is commonly used in the Food Industry to produce snacks. The physicochemical and nutritional properties can be changed during extrusion due to high temperature, pressure and mechanical forces.

Snacks based on a pea protein concentrate (PP), a pea starch concentrate (PS) and an oat beta-glucan-rich fraction (OF) was produced through low-moisture extrusion (LME) **[1]**. The snacks contained 3.5 g of β-glucan per portion. This was high enough for both approved EFSA health claims: lowers cholesterol and reduces post-prandial glucose response. Moreover, Portuguese products, such as, Bravo de Esmolfe apple (BE), grass pea (GP) and a red seaweed (Gracilaria gracilis- GG) were added to the main formulation. The impact of LME on the phenolic composition of the free and bound fractions **[2]** and the antioxidant capacity **[3]** of the blends (non-extruded) and snacks (extruded blends) were evaluated.

Results showed that p-hydroxybenzoic acid and hydroxycinnamic acids were largely provided by OF and the higher kaempferol content was supplied by the PS. The bound fraction presented more than 50% of the hydroxycinnamic acids and p-hydroxybenzoic acid. Moreover, the addition of Bravo de Esmolfe apple (BE) contributed with higher contents of p-hydroxybenzoic acid, caffeic acid, quinic acid, catechin, procyanidin B1, 3-hydroxybenzoic acid and phloridzin, leading to an improvement of the antioxidant capacity (35 µmol TEAC/g dm) of the healthy snacks.

Overall, PS and OF were the main contributors to the phenolic composition of the snacks and in the respective blends prior to extrusion processing. Moreover, extrusion processing didn't change the total phenolic content of the snacks.

Acknowledgments:

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P 049 VALIDATION OF SPECTROPHOTOMETRIC ASSAYS FOR DETERMINING THE ANTIOXIDANT CAPACITY OF NATURAL EXTRACTS AND STANDARD POLYPHENOLS

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Introduction The metal chelating activity, radical scavenging ability, and reducing power contribute to the antioxidant potential of natural antioxidants. According to this theory, it is essential to optimize and validate current protocols to establish a reliable method for evaluating the antioxidant capacity of various natural antioxidants. A growing pressure exists on reducing the use of synthetic ingredients in food, cosmetics, etc., highlighting the importance of natural alternatives.

Material and methods:

This study presents optimized and validated standard procedures for assessing the radical scavenging activity, reducing capacity, and metal chelating activity using the DPPH and ABTS-RSA, FRAP, and ferrozine iron-chelating tests, respectively. These tests were employed to determine the antioxidant potential of natural extracts, including applewood, rosemary, green tea, and grapevine extracts. The potential was compared with synthetic stabilizers in the form of BHT, ascorbic acid, trolox, and EDTA. Furthermore, the natural extracts are subjected to an UPLC screening to identify the present polyphenols. The identified polyphenols are screened using the optimized standard procedures for the determination of their antioxidant potential. As a result, the antioxidative mechanism of individual polyphenols can be framed.

Results:

The validation of the spectrophotometric tests involves evaluating statistical parameters including precision (repeatability and intermediate precision), repeatability, linearity, IC-values and TEC-values. The results show that the validated assays have provided a more reproducible analytical method, limiting statistical variability across days of analysis. Moreover, the study reveals the antioxidant functions of each polyphenol as well as their effectiveness.

Conclusion:

The validated assays presented improved reproducibility, reducing the statistical deviation several days of analysis. Furthermore, the study highlights that certain polyphenols demonstrate antioxidant capabilities comparable to BHT, Trolox, and ascorbic acid. The effectiveness of natural extracts in exerting their antioxidant function depends on the specific polyphenols present in the extract. These findings emphasize the importance of identifying and understanding the composition of polyphenols in natural extracts to optimize their antioxidant potential.

P 050 DEVELOPMENT OF A RAPID UHPLC METHOD FOR ACCURATE PROFILING AND QUANTIFICATION OF 35 POLYPHENOLIC COMPOUNDS IN NATURAL EXTRACTS

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Introduction:

This study presents the validation of a rapid UHPLC method for quantifying polyphenols in natural extracts. Polyphenols, derived from various plants and fruits, exhibit diverse biological effects due to their aromatic ring structures and hydroxyl groups, providing health benefits such as cardiovascular protection, anti-inflammatory properties, antioxidative and antimicrobial activities. The research includes an optimization of the chromatographic parameters for efficient analysis, a complete method validation for UHPLC and quantification of 35 polyphenolic compounds within a timeframe of less than 20 minutes.

Material and methods:

An Agilent 1290 Infinity II UPLC system was used for the identification and quantification of polyphenols in natural extracts. The eluents utilized for the validation of the UHPLC method include methanol, acetonitrile and Milli-Q water, all acidified with formic acid (0.1%). The standards are selected based on their prevalence in natural sources and literature. Daidzein is used as an internal standard due to its rare occurrence in nature.

Validation:

A mixture containing 35 polyphenolic standard components is used to optimize the chromatographic parameters: eluent gradient, temperature gradient and flow rate (mL/min). The selection of the optimal chromatographic separation relies on the optimization of next parameters: retention time, resolution, peak area, peak width, and selectivity. The validation of the UHPLC method involves evaluating statistical parameters including linearity, precision (repeatability and intermediate precision), LOD and LOQ, robustness, and repeatability.

Results and conclusion:

The method demonstrated successful application across various natural extracts, enabling the analysis and quantification of key phenolic compounds in waste streams. Additionally, high reproducibility and intermediate precision values (CV < 5%) were achieved for the retention time, peak area, peak width, and peak resolution. The optimal conditions for achieving this effective separation included a column working temperature of 40 °C, a flow rate ranging from 0.2 to 0.5 mL/min and the utilization of methanol, Milli-Q, and acetonitrile acidified with formic acid.

P 051 DIETARY FLAVONOID INTAKE AND ABDOMINAL AORTIC CALCIFICATION: FINDINGS FROM A COHORT OF ELDERLY WOMEN

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7 *Danish Cancer Society, Danish Cancer Society Research Centre, Copenhagen, Denmark*
8 *The University of Sydney, Centre for Kidney Research, Sydney, Australia*

Background:

Higher habitual dietary flavonoid intake is associated with lower risk of cardiovascular disease (CVD), but the possibility of an association with abdominal aortic calcification (AAC)—a major predictor of vascular disease events—has not yet been investigated.

Objectives:

This study aimed to examine associations between intakes of 1) total flavonoids, 2) flavonoid subclasses, and 3) specific flavonoid-containing foods with AAC in participants from the Perth Longitudinal Study of Ageing Women.

Methods:

Cross-sectional analyses were performed on 881 females (median [IQR] age, 80 [78–82] years; BMI, 27 [24–30] kg/m^2) who had never had a prior CVD event. We used the US Department of Agriculture flavonoid databases to estimate flavonoid intake from food frequency questionnaires. Those with extensive calcification of the abdominal aorta (AAC24 ≥6) were identified using lateral lumbar spine images and logistic regression was used to investigate relationships with our exposures.

Results:

Compared to participants with low intakes (Q1) and after multivariable adjustment for demographics and lifestyle factors, those with the highest consumption (Q4) of total flavonoids, flavan-3-ols, flavonols and flavanones had 37% [OR (95% CI): 0.63 (0.43, 0.92)], 38% [0.62 (0.41, 0.93)], 40% [0.60 (0.41, 0.87)], and 31% [0.69 (0.49, 0.97)] lower odds of extensive AAC, respectively. Except for flavanones, these associations remained significant after additional adjustment for dietary confounders (**Figure 1, Panels A–D**). Intakes of anthocyanins and proanthocyanidins showed no compelling associations with AAC. In food-based analyses, a higher intake of black tea, but not chocolate, red wine or fruit juice, was associated with significantly lower odds of extensive AAC [participants consuming 2–6 cups black tea/day had a 16–42% lower odds compared to non-consumers (**Figure 1, Panel E**)]. Given the major source of total dietary flavonoid intake was black tea (75.9%), to disentangle the impact of tea and non-tea flavonoids on AAC, we conducted analysis on a subset of participants who did not consume black tea. In this subgroup, higher total non-tea flavonoid intake beneficially associated [Q4 vs. Q1 OR (95% CI): 0.13 (0.03, 0.49)] with lower odds of extensive AAC (**Figure 1, Panel F**).

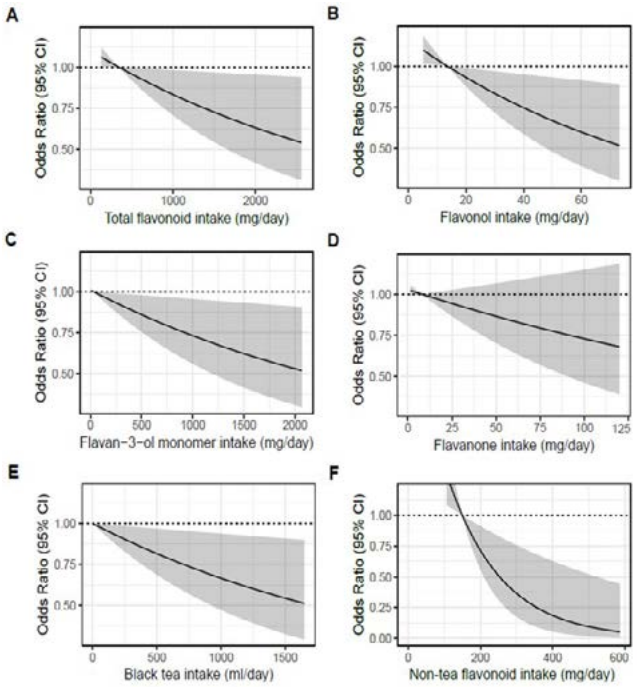

Figure 1. Panels depict the association of A) total flavonoid intake B–D) selected flavonoid subclasses E) black tea consumption and F) total flavonoid intake in those who don't drink black tea, with the odds of extensive abdominal aortic calcification (AAC; AAC 24-point scoring method [AAC24] ≥6). Odds ratios are comparing the specific level of flavonoid intake (horizontal axis) to the median intake for participants in the lowest intake quartile. Adjusted for age, body mass index (BMI), smoking, energy expended in physical activity, alcohol intake, antihypertensive medication use, statin use, the Calcium Intake Fracture Outcome Study treatment code and intakes of dietary energy, saturated fat, polyunsaturated fat, monounsaturated fat, sodium, and fiber.

Conclusions:

Greater habitual dietary flavonoid intake associates with a lower propensity of the abdominal aorta to calcify. These results suggest that populations with low black tea consumption and otherwise low flavonoid intake may benefit by increasing intake of tea or non-tea derived flavonoids.

P 052 DIETARY POLYPHENOLS IN THE MODULATION OF COW'S MILK ALLERGY

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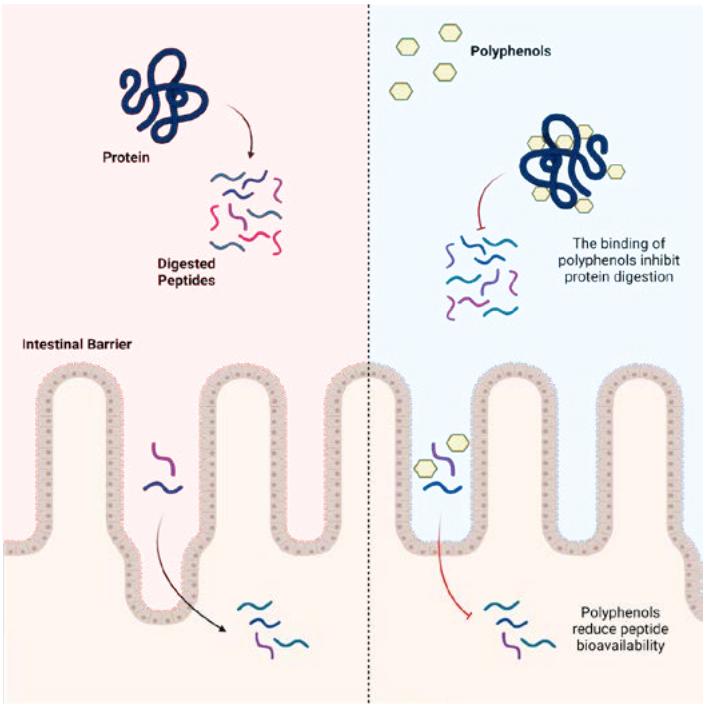
Background:

The rapid increase in the prevalence of food allergies is emerging as a new challenge among nutritionists, clinical and food researchers, and the food industry. Among them, cow's milk allergies have prompted the need for the development of new therapeutic strategies to prevent this pathology and reduce its symptoms. The use of dietary phenolic compounds to modulate the digestion of allergens constitutes an exciting new approach for maintaining oral tolerance to allergens. Phenolic compounds are a class of plant-derived bioactives with a myriad of described biological activities. These compounds can also bind to different dietary proteins, which can alter not only their digestion pattern but also modulate their bioavailability. As such, the use of phenolics as modulators of oral tolerance to dietary allergens has been proposed as a new strategy to prevent allergic reactions to potentially allergenic foods.

Figure1: Effect of polyphenols on the digestion of proteins and the bioavailability of peptides.



Objectives:

The main objectives of this work were thus to monitor the effect of dietary polyphenols present in green tea and blueberries on the bioavailability and bioaccessibility of potentially allergenic milk peptides. Green tea and blueberry were chosen for these experiments as they are rich in flavanols and anthocyanins, respectively. Methods: Both milk proteins and dietary polyphenols were extracted and characterized. After that, *in vitro* digestion models were used to simulate the ingestion and digestion of foods, and mass spectrometry techniques were used to track the potentially immunogenic peptides during the digestion process. Samples were digested simultaneously with extracted green tea polyphenols and blueberry polyphenols to pinpoint the potential effects of different phenolic compounds on the digestion of milk proteins and the formation of immunogenic peptides. To evaluate the effect of polyphenols on the bioavailability of different immunogenic peptides, samples resulting from the different digestion experiments were incubated on the apical side of a monolayer of intestinal epithelial cells (Caco-2) to recreate the dynamics of the uptake of allergens at the intestinal barrier. The intestinal uptake of immunogenic peptides was monitored using mass spectrometry techniques.

Results:

Regarding the effect of polyphenols on bioaccessibility, digestion of milk proteins with both of the polyphenol extracts resulted in a lower final concentration of some of the monitored immunogenic peptides compared to samples digested without polyphenols or with the extract without polyphenols. Likewise, the bioavailability of some of the monitored peptides was lower when they were incubated with the dietary polyphenols. However, none of the monitored peptides were detected on the basolateral side of the epithelial monolayer, indicating that they were hydrolyzed either on the apical side or inside epithelial cells.

Conclusion:

These results have demonstrated that phenolic compounds present in both green tea and blueberry can significantly affect the bioaccessibility and bioavailability of cow's milk allergy-related peptides. The role of brush-border enzymes and the extended metabolization inside intestinal epithelial cells was also highlighted in the bioavailability of immunoreactive peptides. This study opens a new way to better understand the nutritional role of polyphenols in cow's milk allergy.

P 053 ENZYMATIC SYNTHESIS OF SULFATED METABOLITES OF PHENOLIC COMPOUNDS

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Polyphenols are the most abundant natural substances found in plant foods. The largest polyphenol groups are flavonoids, phenolic acids and catechols. Polyphenols are metabolized in the II phase of biotransformation, where they are mainly sulfated or glucuronidated. The synthesis of sulfated metabolites could help to understand the biotransformation of polyphenoles and their effects on the human body. Isolation of these metabolites from biological material is impractical, but the metabolites can be synthesized *in vitro*. Sulfated polyphenols can be prepared by enzymatic sulfation, which requires much milder reaction conditions than chemical sulfation. Bacterial arylsulfotransferases (ASTs) are a convenient tool for this purpose. The recombinant AST from *Desulfitobacterium hafniense* is easily to produce and uses an affordable phenolic substrate *p*-nitrophenyl sulfate (*p*-NPS) as a sulfate donor [1]. In the present study, potential recombinant ASTs were produced in the microbial expression system of *E. coli*. The produced enzymes were purified and characterized, and their kinetic parameters were determined using phenol and catechol as sulfate acceptors. Substrate specificities were tested using *p*-NPS as sulfate donor and various flavonoids as acceptors. To reliabley compare the impact of the acceptor structure, the chosen acceptors differed mainly in the amount and positions of the-OH groups. Namely, sulfation abilities of selected enzymes were tested with chrysin, apigenin, luteolin, kaempferol, myricetin, quercetin and silydianin. The reactions were monitored using HPLC methods optimized for the proper separation of polar and highly polar substances in the reaction mixture [2].

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P 054 ANTHOCYANINS FROM PURPLE CORN CAN PREVENT THE PROGRESSION OF MULTIPLE SCLEROSIS AND ASSOCIATED TRIGEMINAL PAIN: EFFECT ON NEUROINFLAMMATION AND AUTOPHAGY

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Background:

Anthocyanins (ACNs) are bioactive constituents of fruits and vegetables that provide health benefits and are able to prevent pathologies, such as cardiovascular and age-related neurodegenerative diseases, and metabolic disorders. In fact, ACNs have specific anti-inflammatory, antioxidant properties and neuroprotective effects [1]. The administration of ACN-enriched purple corn extracts is able to reduce trigeminal-associated pain in various pathological conditions, also thanks to the modulation of microglia reactivity [2].

Objectives:

Aim of this study was to examine whether ACN-rich purple corn dietary supplement has positive effects on trigeminal pain associated to multiple sclerosis (MS) and on the onset and progression of MS.

Methods:

Eleven days before the induction of experimental autoimmune encephalomyelitis (EAE) [3], Dark Agouti rats were assigned to drink water, yellow or purple corn extracts. Yellow corn extract is isogenic to the purple one, but does not contain ACNs, and is therefore utilized as negative control. The development of EAE and TG pain was evaluated up to 21 days post EAE induction, and fecal samples were collected at significant time points for the analysis of microbiota composition and ACN metabolites. After sacrifice, central nervous system tissues were collected for qPCR, ELISA and WB analyses.

Results:

Results show that, thanks to gut ACN metabolism and modifications of the microbiota, purple corn positively influences the progression of EAE motor symptoms and protects from associated TG pain by modulating glia activation, the expression of pro-/anti-inflammatory mediators and autophagy.

Conclusions:

Our findings suggest a possible application of purple corn extract as nutraceutical supplement to be administered as co-adjuvant to pharmacological treatments against MS and associated symptoms.

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P 055 IMPACT OF THERMAL AND NON-THERMAL TECHNOLOGIES ON CHEMICAL-PHYSICAL AND MICROBIOLOGICAL PROPERTIES, AND BIOACTIVE COMPOUNDS OF STRAWBERRY SMOOTHIES

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Enhancing the shelf-life of fruit and vegetables products is an outstanding field of research for food industry and, at the same time, consumers are in search of minimally processed food with minimal losses in terms of nutritional and healthy composition. Smoothies are an alternative to filtered juices or highly processed beverages, and high pressure processing (HPP) has recently gained attention as a no-thermal technology that has aimed to reduce the microbiological load while preserving the properties of juices. So, elaborated strawberry smoothies were treated by HPP and compared with a conventional thermal pasteurized (TP) and non-treated (NT) smoothies focusing on their chemical-physical (pH, °Brix, colour and viscosity) and microbiological properties (mesophilic bacterial count (MBC), yeasts and molds (Y&M), Enterobacteriaceae (Ent.) and anaerobic bacteria (AB)), and bioactive compounds. Phenolic compounds were analysed by HPLC-ESI-TOF-MS, in negative and positive mode, the vitamin C content by HPLC-UV/VIS, and the antioxidant activity was determined by DPPH and ABTS assays. The HPP process was able to preserve the colour of the smoothies, whereas no significant differences were observed for pH and °Brix. The viscosity of HPP samples were higher respect to NT or TP samples. All the samples showed MBC, Y&M, Ent. and AB < 1 Log CFU/ml. A total of 19 phenolic compounds and precursors, and 5 anthocyanins were identified and quantified. The TP smoothie showed significative (p<0.05) reductions in all the bioactive compounds analyses compared with NT. In the polyphenols significative increments of 18, 12 and 16 % were observed in the sum of phenolic acids, sum of flavonoids and sum of all phenolic compounds, respectively, in the HPP treated compared with NT. In the anthocyanins, as are the more sensitive compounds, significative (p<0.05) reductions were showed by the two treatments but lower in the case of HPP (32%) compared with TP (55%). Regarding the vitamin C content, the HPP treated exhibited a significative (p<0.05) increment in the ascorbic acid and a significative (p<0.05) reduction in the dehydroascorbic acid content increasing the AA/DHAA ratio from 6.9 in NT to 9.2 in the HPP treated, and 15% the total vitamin C content significatively (p<0.05). For DPPH no significative (p<0.05) changes were appreciated between NT and HPP samples, but a significative (p<0.05) increase of 12% was seen by ABTS assay in the HPP. Besides a Pearson correlation analysis showed that the sum of phenolic compounds, specifically phenolic acids, and the vitamin C content, specifically ascorbic acid, were the compounds that had the highest significant correlation with the antioxidant assays. So HPP has demonstrated to preserve the colour and increase the bioactive compounds content of strawberry smoothies increasing their antioxidant activity being a better technology for processing than a conventional thermal treatment.

P 056 ASSESSMENT OF HUMAN INTER-INDIVIDUAL VARIABILITY OF PHLORETIN METABOLITES IN URINE AFTER APPLE CONSUMPTION

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Background:

Heterogeneity in the pharmacokinetic response after (poly)phenol intake has been commonly observed in human clinical trials, and this fact can hinder the health beneficial effects in some sub-populations. Although phloretin phase-II conjugates have been reported as specific biomarkers of apple intake, investigation is lacking regarding the inter-individual variability after a repeated exposure.

Objectives:

This study is aimed to assess the inter-individual variation in phloretin absorption and metabolism following apple snack consumption and to seek possible phloretin metabotypes determining whether the metabolite excretion profiles were temporally consistent, varied with phloretin dose, or varied after stratification.

Methods:

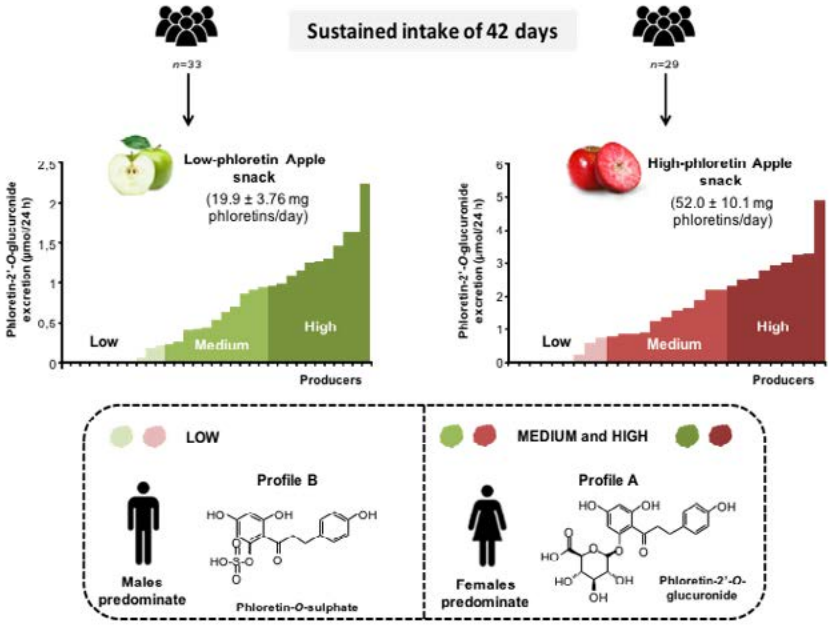
The study consisted of a subsample of the AppleCOR study. The excreted phloretin metabolites in 24-h urine samples were determined by UPLC-MS/MS in 62 volunteers after acute and sustained (6 weeks) interventions in a randomized, controlled and parallel study with a daily supplementation of 80 g of a low-phloretin (Low-PhA; 39.5 µmol; n=33;) or a high-phloretin (High-PhA, 103 µmol; n=29) freeze-dried apple snacks. Nested within the sustained study, a sub-sample of volunteers of each group (*n*=7, Low-PhA; *n*=8, High-PhA) performed two acute intake studies, the first occurred at day 0 and the second occurred at the end of the sustained study.

Results:

Overall, absorption estimated as phloridzin equivalents for 62 volunteers varied almost 70-fold ranging from 0.1% to 6.94% of phloretin glycoside intake reflecting pronounced inter-individual differences. Volunteers were stratified into low, medium and high producers and by the balance between glucuronidation and sulphation observing two groups depending on whether glucuronide conjugation (A) or sulphate conjugation (B) dominated in urine. Overall glucuronidation dominated with sulphation, prominent only among low producers. Analysis of plasma glucose and insulin at the start and end of the 6-week sustained study showed a trend towards modest reductions for high producers, suggesting higher potential benefits for those individuals capable of greater phloretin absorption. It is also discussed the plausibly factors contributing to the inter-individual variation in phloretin uptake, such as variations in the hydrolysis of phloretin glycosides by lactase-phloridzin hydrolase (LPH) in the oral microflora or the lactase non-persistence trait.

Conclusions:

This study demonstrates clearly a marked inter-individual variation in the excretion of phloretin phase-II conjugates following consumption of apple snacks. There were inconsistent effects on post-prandial plasma glucose and insulin concentrations but there was a tendency for decreases in these parameters to be associated with higher excretion of phloretin phase-II conjugates, so further investigations are justified to identify apple phloretin absorption-limiting mechanisms. It would be advantageous if future volunteers were screened for lactose intolerance and the phloridzin-hydrolysing activity of their oral bacteria, as they are plausibly factors contributing to the reported substantial inter-individual variation in phloretin uptake.


Sustained intake of 42 days
n=33
n=29
Low-phloretin Apple snack
(19.9 ± 3.76 mg phloretins/day)
High-phloretin Apple snack
(52.0 ± 10.1 mg phloretins/day)
Phloretin-2'-O-glucuronide excretion (µmol/24 h)
Low
Medium
High
Producers
LOW
MEDIUM and HIGH
Profile B
Profile A
Males predominate
Females predominate
Phloretin-O-sulphate
Phloretin-2'-O-glucuronide

P 057 BIOACTIVE COMPOUNDS FROM OLIVE POMACE: *IN VITRO* DIGESTION MODELS TO ASSESS THE BIOACCESSIBILITY OF PHENOLIC COMPOUNDS

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Background:

Olive pomace (OP) is a by-product obtained from the olive oil extraction process. Their phenolic compounds have been characterized since they have been related to health benefits. Despite that the phenolic concentration varies depending on factors such as olive variety, oil extraction process, and storage conditions, this by-product is generating great interest to be used as an alternative source of these phytochemicals, however, they must be bioaccessible and bioavailable to exert their bioactive effects.

Objectives:

We aimed to conduct a literature review of the current *in vitro* static digestion methods applied to OP to evaluate the effects and bioaccessibility of phenolic compounds.

Methods:

Article selection was based on a literature review using the electronic databases Pubmed, Scopus, and Web of Science. Searches were made using combinations of the terms *olive pomace,* in vitro *digestion, polyphenols, and bioaccessibility.* The selection of scientific articles was performed by either verifying the title and abstract or by reading the full text. Duplicate publications were excluded, and the resulting ones were scanned to retrieve additional relevant research.

Results:

Different *in-vitro* digestion methods differ mainly by the number and type of steps involved in digestion (e.g., mouth, stomach, small intestine), the composition of digestive fluids used in each step (e.g., enzymes, salts, buffers), as well as experimental conditions that may affect enzyme activity (such as enzyme to substrate ratios, pH, temperature, or duration of digestion). Using the standardized Infogest method (1), Radick et al. (2) determined that 3-hydroxytyrosol, tyrosol, and oleuropein are maintained in constant amounts during gastrointestinal digestion. They found that only tyrosol exhibited an increase in all bioaccessible fractions of the samples. The method of Minekus et al. (3) used to evaluate the effect of olive pomace micronization on bioaccessibility, showed that particle size reduction increases bioaccessibility since it seems that the release of polyphenols in the salivary and gastric phases is augmented (4). The phenolic compounds of a pulp-enriched powder obtained from olive pomace were shown to be bioaccessible in significant amounts (5). An *in vitro* digestion method in combination with a dialysis process was used. Despite the fact that the presence of food in the digestive system may slightly improve the bioaccessibility of other OP polyphenols, changes in pH, enzyme activity or bile do not appear to be determinants of the bioaccessibility of hydroxytyrosol and tyrosol (6–8).

Conclusion:

In vitro digestion methods are useful for evaluating the bioaccessibility and bioavailability of bioactive compounds in olive pomace. These findings suggest that further optimization of olive pomace processing methods could enhance the bioaccessibility of its bioactive compounds.

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P 058 BITTER TASTE RECEPTOR'S EXPRESSION THROUGH THE GASTROINTESTINAL TRACT OF WISTAR RATS

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Obesity is a multifactorial disease that is rising quickly in developed countries. This increase is mainly due to the consumption of western-style diet. This diet consists mainly in refined sugars, saturated fats and highly energetic foods with low nutritional value. Chronic consumption of this diet induces intestinal disturbances and affects the organism's metabolism. Grape seed proanthocyanidin extract (GSPE) has natural bioactive compounds which proved to have a protective effect against the effects of an obesogenic diet. One of the postulated pathways by which GSPE exerts its effects is through the activation of extraoral bitter taste receptors (Tas2r). Our aim is to determine the expression profile of Tas2r through the gastrointestinal tract of rats and to reveal the possible effects of a cafeteria diet and / or GSPE treatment. Rats were fed a 17-week standard (STD) or a cafeteria diet (CAF) with or without oral-GSPE supplementation at 500 mg GSPE/kg body weight: 10-days preventive treatment (PRE-CAF), or final 10-day GSPE administration (CORR-500). qPCR analyses were performed to assess the gastrointestinal profile of Tas2r. As previously seen, it was proved that the expression of bitter taste receptors is extremely low in rats. Furthermore, it was observed that each intestinal tract possesses a characteristic expression profile. Cafeteria diet reduces the expression of Tas2r in most intestinal segments. On the other hand, GSPE administration (correctively and preventively) also modulated these expressions. In conclusion, Tas2rs are postulated as a new mechanism by which bioactive compounds modulate different intestinal functions and therefore systemic metabolism.

P 059 WHEN DIVERSITY IN THE HALOPHYTE SPECIES MEANS PHENOLIC COMPOUNDS VARIABILITY

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Halophytes, known for their ability to grow under adverse saline conditions, above 200 mM of sodium chloride, can be cultivated using soilless cultivation systems (SCS) and have been pointed out as an alternative to salt to prepare meals. These plants may be also an important source of phenolic compounds. SCS approach has been applied in Portugal to improve halophytes' productivity and nutritional quality. However, there is a lack of knowledge concerning the impact of SCS in the phenolic composition of halophyte species. In this study, seven distinct halophyte species cultivated by SCS (*Disphyma crassifolium* L., *Crithmum maritimum* L., *Inula crithmoides* L., *Mesembryanthemum crystallinum* L., *Mesembryanthemum nodiflorum* L., *Salicornia ramosissima* J. Woods, *and Sarcocornia fruticosa* (Mill.) A. J. Scott.) were studied for their phenolic composition. Extracts were prepared using conventional solvent extraction (ethanol: water, 80:20, v/v) and phenolic compounds were analysed by HPLC-DAD-ESI-MS/MS for target and non-target analysis. The total phenolic content was also measured using Folin Ciocalteau's method.

The different species were characterized by distinct families of phenolic compounds. *C. maritimum*, *S. ramosissima* and *S. fruticosa* showed the highest content in phenolic acids, namely hydroxycinnamic acids. The three species with the highest content of flavonoids, were *S. fruticosa*, *C. maritimum*, and *D. crassifolium*. While in *D. crassifolium* flavone compounds such as rutinoside, and glucoside derivatives of isorhamnetin were identified, in *I. crithmoides*, the vitexin (apigenin 8-C-glucoside) was one of the unique compounds detected in this species. The flavanones pinobanksin-5-methyl-ether-3-O-acetate and rhamnetin were also examples of typical compounds in *I. crithmoides*. The presence of flavones such as apigenin 6-C-glucoside or apigenin 6-C-glucoside-7-O-glucoside and diosmetin 7-O-rutinoside was characteristic of *C. maritimum*. Other compounds such as the flavanonol dihydroquercetin and the flavanone rhamnetin hexosyl pentoside were only detected in *S. fruticosa*, and the hydroxycinnamic acids, ferulic acid, 3,4- and 4,5-dicaffeoyl quinic acids only detected in *S.ramosissima*. Compounds such as 3,5-diferuloylquinic acid, eriodictyol, and epicatechin hydrate, were detected in *M.nodiflorum* and the presence of sinapic acid glucoside and kaempferol was noted in *M. crystallinum*. AS a conclusion not only qualitative differences were detected, but there were also quantitative differences in the compounds commonly quantified in the different species. As an alternative form of salt, these plants were added to popcorns, *ketchup*, and soups with a good sensorial acceptance.

Acknowledgments:

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P 060 AN ANTHOCYANIN-RICH EXTRACT FROM PORTUGUESE BLUEBERRIES AS A NOVEL STRATEGY FOR THE MANAGEMENT OF AUTISM SPECTRUM DISORDERS: AN *IN VIVO* MODEL

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3 *Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*
4 *Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal*
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6 *Department of Life Science, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal*

Background:

Anthocyanins are compounds found in high concentrations in blueberries, which have been envisaged as promising therapeutical agents in the context of many brain disorders, thanks to their potent anti-inflammatory, antioxidant, and immunomodulatory effects.

Autism Spectrum Disorders are complex neurodevelopmental conditions the pathophysiology of which is not yet fully understood. Since the available current treatments are not curative and remain largely ineffective, it is still imperative to find novel strategies to manage these disorders.

Objectives:

This study aims to investigate the ability of an anthocyanin-rich extract (ARE) obtained from Portuguese blueberries to regulate the microbiota-gut-brain axis in a valproic acid (VPA) mouse model, contributing, in this way, to the management of autistic-like behaviours.

Methods:

Pregnant BALB/c females were treated subcutaneously with a single dose of VPA (500 mg/kg) or saline on gestational day 12.5. Male offspring mice were orally treated with the ARE from blueberries (30 mg/kg/day) or the vehicle for three weeks, and further subjected to behavioural tests and biochemical analysis.

Results:

Our data suggested that ARE treatment was successful in reducing neuroinflammation and gut inflammation, in regulating gut microbiota composition and in alleviating autism-like behaviours in *in utero* VPA-exposed mice.

Conclusion:

The anthocyanins extracted from blueberries could constitute an innovative strategy in the context of autism spectrum disorders, via modulation of the microbiota-gut-brain axis.

This work was funded by COMPETE 2020 and by Portuguese national funds via FCT, grants POCI-01-0145-FEDER-029089/ PTDC/SAU-NUT/29089/2017 and LISBOA-01-0145-FEDER-402-022125.

P 061 HEALTH-PROMOTING PROPERTIES OF SWEET CHERRY EXTRACTS

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Phenolic compounds are present in natural-based foods and they have been a focus of much research owing to their ability to reduce oxidative stress levels once it is implicated in multiple diseases and inflammatory conditions. These benefits are mainly due to their chemical structure, composed of hydroxyl groups, which confers powerful capacity to scavenge free radicals and reactive species and to increase the activity of detoxifying enzymes. Among fruits, sweet cherries have been a target of many studies given their richness in phenolic compounds. Given these facts and taking into account that sweet cherries are largely produced in Portugal, particularly in Fundão region, we decided to study one of the most appreciated sweet cherry cultivars, which is Saco, regarding their phenolic composition through using HPLC-DAD-ESI/MS^n, and evaluate the antioxidant effects against ferric and peroxide species, as well as superoxide and nitric oxide radicals, and inflammatory properties of its enriched phenolic extracts on lipopolysaccharide-stimulated RAW 264.7 macrophages. A total of 4 anthocyanins and 35 non-coloured compounds were detected. As expectable, cyanidin 3-*O*-rutinoside and chlorogenic acids were the predominant ones. Focusing on the biological potential, phenolic-rich extracts of Saco (50–800 µg/mL) showed capacity to reduce ferric species and superoxide and nitric oxide radicals in a dose-dependent manner. Regarding ferric species, the coloured fraction was the most efficient (IC_{50}=9.43±0.43 µg/ml), while the total extract was the most active in neutralizing peroxide species (IC_{50} value of 56.39±1.26 µg/mL of dw). Regarding nitric oxide and superoxide radicals, the total extract was also the most effective (IC_{50} score of 33.72 ± 0.89 and 41.68 ± 0.72 µg/mL of dw, respectively). In addition, all fractions also showed capacity to interfere with cellular nitric oxide (NO) levels by capturing NO radicals and decreasing inducible nitric oxide synthase and cyclooxygenase-2 expression. The present data reinforces the idea that sweet cherries can be incorporated into new pharmaceutical products, smart foods and nutraceuticals and be effective in preventing and/or treating diseases mediated by inflammatory mediators, reactive species and free radicals.

P 062 HIGHER HABITUAL INTAKES OF FLAVONOIDS AND FLAVONOID-RICH FOODS ARE ASSOCIATED WITH A LOWER INCIDENCE OF TYPE 2 DIABETES IN THE UK BIOBANK COHORT

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3 *Edith Cowan University, Nutrition & Health Innovation Research Institute, School of Medical and Health Sciences, Joondalup-Western Australia, Australia*
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5 *Institute of Health Carlos III, Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición CIBEROBN, Madrid, Spain*
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Objective:

To examine the associations between habitual intakes of flavonoid sub-classes, the major food contributors to flavonoid intake, and a novel FlavoDietScore (FDS) and incident type 2 diabetes (T2D) in a large prospective cohort of middle-aged adults.

Research design and methods:

Adult participants from the UK Biobank cohort (≥2 24-h dietary recalls) were included. Cox proportional hazard regression models were conducted to estimate hazard ratios (HR) and 95% confidence intervals (CI) for associations between intakes of the FDS (which reflects consumption of flavonoid-rich foods), the individual food contributors to flavonoid intake, flavonoid subclasses, and T2D. Mediation analyses based on biomarker measurements were carried out to assess potential biological mechanisms.

Results:

In this study of 120,923 UK Biobank participants, 2,951 incident T2D cases were identified during 11.8 years of follow-up. Mean (SD) age was 56.0 (7.8) years and 56.6% of the participants were female. A higher FDS (highest vs lowest quartile) was associated with a significantly lower risk of T2D (hazard ratio (95% confidence interval) 0.87 (95% CI: 0.78–0.97) following multivariate adjustment. This association was equally observed among people with medium and high genetic risk. In mediation analyses, lower basal inflammation (lower levels of C-reactive protein) was the strongest mediator of associations between FDS and T2D risk, with a proportion mediated of 8%. Further mediators with proportions mediated of 7% were better kidney (lower cystatin C) and liver function (lower gamma-glutamyl transferase and alanine aminotransferase). Intakes of most flavonoid subclasses (anthocyanins, flavan-3-ols, flavonols, flavones, polymers, and proanthocyanidins), except flavanones, were associated with a significantly lower risk of T2D (Q4 vs. Q1) with HRs (95% CI) of 0.79 (95% CI: 0.70–0.88), 0.84 (95% CI: 0.76–0.94), 0.80 (95% CI: 0.72–0.90), 0.84 (95% CI: 0.76–0.94), 0.85 (95% CI: 0.77–0.94), 0.81 (95% CI: 0.73–0.90), and 0.98 (95% CI: 0.89–1.09), respectively.

Conclusions:

Consumption of flavonoid-rich foods is associated with lower T2D risk, possibly mediated by beneficial effects of inflammation, kidney function, and liver function. Encouraging an achievable increase in habitual intake of specific flavonoid-rich foods and beverages, namely tea, berries, apples, and red wine may lower T2D risk.

P 063 PIGMENTED UPLAND POTATOES: DIFFERENT *IN VITRO* ANTI-INFLAMMATORY ACTIVITY BASED ON THEIR DISTINCTIVE BIOACTIVES

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Background:

In a climate change scenario, finding resilient crops able to grow with fewer resources, but still nutritionally relevant and with potential beneficial properties, has become of utmost importance. In this context, pigmented upland potatoes could represent a valid sustainable source of bioactive compounds for the future. Since the pigments that characterize these varieties are naturally occurring anti-inflammatory and antioxidant compounds, such as carotenoids and anthocyanins, these tubers may serve as functional food to prevent non-communicable diseases (NCDs) whose risks factors are, among others, unhealthy diet and consequent chronic inflammation. In fact, carotenoid and anthocyanin consumption inversely correlates with the development of chronic diseases, such as obesity, cancer, neurodegenerative and cardiovascular diseases by counteracting inflammation.

Objectives:

We aimed to study the anti-inflammatory activity of different combinations of phytonutrients present in three upland potato varieties proposed as a possible source of bioactive compounds.

Methods:

Tubers from three commercial potato varieties differently enriched in phytonutrients, named Kennebec (yellow-skinned and white-fleshed), Desirée (red-skinned and yellow-fleshed) and Bleuet (purple skinned and fleshed) have been cultivated on Italian mountains (Starleggia, Valle Spluga, Lombardy, 1560masl). Extracts from lyophilized whole tubers have been obtained and analyzed through HPLC-DAD and spectrophotometric analysis. THP-1 human monocytes were differentiated in macrophages through PMA-treatment, treated with the three extracts and then challenged with LPS to induce an inflammatory response. The dose-dependent effects of the different combinations of phytonutrients on pro-inflammatory genes and/or proteins were evaluated.

Results:

The extracts from Kennebec, Desirèe and Bleuet were found to be enriched in clorogenic acid (CGA), carotenoids and anthocyanins, respectively. All three extracts were able to counteract LPS-induced inflammation in THP-1 macrophages when provided at higher doses. Nevertheless, at lower doses, comparable with those detected in human plasma after potato consumption, only the carotenoid- and anthocyanin-rich extracts from Desirèe and Bleuet exerted a significant anti-inflammatory activity. A further analysis of the anti-inflammatory activity of flavonoid fractions suggests a possible interaction among different classes of polyphenols.

Conclusion:

Our results suggest that pigmented upland potatoes, like Desirée and Bleuet, may represent an economical and resilient source of bioactive compounds with the ability to prevent inflammation.

Acknowledgements:

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P 064 FAECAL MICROBIAL METABOLISM OF FERULIC ACID ACROSS VARIOUS AGE GROUPS

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Background:

Ferulic acid (FA) is a major dietary phenolic compound found mainly in coffee, cereals, and other plant-based foods, showing antioxidative, anti-inflammatory effects, and improvement of glycemia and lipid homeostasis. Main metabolism and absorption occur in the lower digestive tract, where colon microbiota releases FA covalently bound to cell wall fiber. The role of FA's effects and interactions with gut microbiota remains unclear.

Methods and Objectives:

We compared the metabolism of a dietary relevant FA concentration of 50 µg/ml *in vitro* using static faecal incubations inoculated with stool of 20 subjects belonging to two age groups (10 young adults under 45 years, and 10 seniors above 70 years old). Samples were taken at time points 0, 2, 4, 8, and 24 hours and used for metabolome and microbiome profiling. The metabolome including metabolites of FA itself was analysed using nuclear magnetic resonance spectrometry (^{1}H NMR), while microbiome was analysed using 16S rDNA Illumina sequencing.

Results:

Five metabolites of ferulic acid were quantified using ^{1}H NMR. The main microbial transient catabolite of ferulic acid was hydroferulic acid, which was further dehydroxylated to 3-phenylpropionic acid. FA was shown to be completely catabolized after 4 hours in the young participants, whereas catabolism was slower in the older group, reaching complete catabolism after 8 hours. Younger subjects also showed higher production of SCFA and utilisation of glucose. FA showed no change in microbiome composition.

Conclusion:

Our study suggests that dietary intake of FA is degraded faster by young subjects compared to seniors and it does not affect microbiota composition or fermentation pathways in the gut. In addition, our study shows the high potential of ^{1}H NMR as a method able to provide a holistic picture of the catabolism of dietary phenolics in *in vitro* models.

Acknowledgment:

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P 066 MILK THISTLE FLAVONOLIGNANS: FROM TRADITIONAL MEDICINE TO MOLECULAR EFFECTS

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Silymarin, an extract from the fruits of milk thistle (*Silybum marianum*), is used in various medicinal applications since ancient times. A major component of silymarin is the flavonolignan silybin and its relatives isosilybin, silychristin, silydianin, 2,3-dehydrosilybin, and several others. With the exception of silydianin, they occur naturally as two stereomers.

This contribution focuses on recent developments in chemistry, biosynthesis, modern advanced analytical methods, and transformations of flavonolignans specifically reflecting their chirality. Recently described chemotypes of *S. marianum*, as well as recent findings on the pharmacokinetics, hepatoprotective, antiviral, neuroprotective, and cardioprotective activity, modulation of endocrine functions, modulation of multidrug resistance, and safety of flavonolignans are discussed.

A growing number of studies show that the respective stereomers of flavonolignans have markedly different activities in anisotropic biological systems. Moreover, it is now clear that flavonolignans do not act as antioxidants *in vivo*, but as specific ligands of biological targets and therefore their chirality is crucial. Controversy frequently arises, mainly due to the nonstandard composition of silymarin-containing phytopreparations, the use of various undefined mixtures, the misclassification of silymarin and silybin, and the failure to consider the chemistry of the respective components of silymarin.

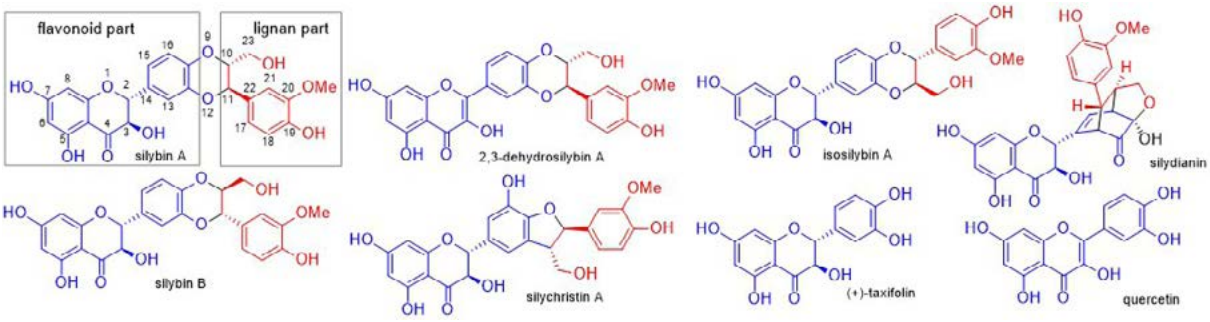

Fig. 1: Silymarin flavonolignans, flavonoid part in blue, lignan part in red.

Reference:

Křen V. & Valentová K. *Nat. Prod. Rep.*, 39, 1264-1281, 2022. http://dx.doi.org/10.1039/D2NP00013J.

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P 067 SIMULATED GASTROINTESTINAL DIGESTION OF FOODS AND BIOACTIVES: SIZE AND CHARGE OF *IN VITRO* PRODUCED MIXED MICELLES

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Background:

Due to the cost and time-consuming nature of *in vivo* research, there is a lot of focus on the *in vitro* digestion and bioaccessibility of bioactives, in particular fat-soluble vitamins and (poly)phenols. Size and surface charge (ζ-potential) of particles in the *in vitro* produced mixed micellar fraction, measured with dynamic light scattering, are increasingly being published. These characteristics are important as they can affect the cellular absorption and transport of the mixed micelles, and the bioactives they encapsulate. However, presentation of micellar size data is not consistent and often limited to only the average particle diameter, making comparisons between different publications difficult.

Objectives:

The first aim was to evaluate different representations of size data (mean particle diameter, relative intensity- or volume-weighted size distribution) to better understand the characteristics of mixed micelles. Secondly, we assessed whether different micellar contents, achieved by digesting different bioactive-rich foods and fat-soluble bioactives, influence the size and surface charge.

Methods:

Bioactives (RRR-α-tocopherol, retinyl-palmitate, βcarotene, curcumin and naringenin) were subjected to a simplified *in vitro* digestion, while foods (spinach and red cabbage) were subjected to both the simplified and the up-to-date INFOGEST 2.0 protocol. Samples were digested with and without olive oil. To obtain the mixed micellar fraction, the digesta were centrifuged (5000 g, 1 h, 4 °C) and filtered (filter cut-off 200 nm). The size and surface charge (ζpotential) of the particles in this mixed micellar fraction was measured using dynamic light scattering.

Results:

The average polydispersity index (PDI) and particle size in the total dataset ranged from 0.143 to 0.637 and 110.7 to 202.6 nm, and respectively. Although the samples were not stored before measurement, we observed broad size distributions (high PDI) and a considerable proportion of particles above the filter cut-off (200 nm), indicating aggregation and dynamic size changes. Addition of oil had a significant but minor effect (- 8.0 nm diameter) on average particle size. The content of the micelles had a significant effect on the micellar size and accounted for 46.1 % of the total size variance (Two-way ANOVA).

All samples had a negative ζpotential ranging from -21.8 to -87.7 mV. Digestion of bioactives resulted in notable lower ζpotential (-59.2 to -87.7 mV) compared to digestion of foods (-21.8 to -72.5 mV). The addition of oil led to more negative ζpotential reducing it by 22.8 mV. The bioactive/food digested and the addition of the oil accounted for 28.1 and 66.1 % of the total ζpotential variance (Two-way ANOVA).

Conclusion:

The polydisperse character of mixed micellar fraction is a challenge in determining the particle size. Thus, reporting the average particle diameter alone can be misleading and should be accompanied by parameters such as polydispersity index and volume-weighted distribution. The negative surface charge of the mixed micelles is even further reduced in the presence of olive oil, indicating higher stability of the mixed micelles.

P 068 APPLEWOOD AS A SOURCE OF VALUE-ADDED COMPOUNDS FOR COSMETIC FORMULATIONS

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Introduction:

The European Green Deal envisions better protection of both people and the environment against harmful substances (European Commission, 2020). In addition, the Green Deal aims at efficient use of resources, with an emphasis on recovering waste streams while targeting sustainability. In this perspective, wood from apple trees that are deemed useless, might in turn become feedstock for the extraction of bioactive compounds applying a green, environmentally friendly technique. The aim of the present study was to evaluate the antioxidant properties of applewood ultrasonic water-ethanol extracts for use as active ingredients in the cosmetic industry. Most cosmetic formulations are oil in water emulsions (O/W) and lipid oxidation is a major cause of quality deterioration.

Objective and methods:

The aim of the present study was to determine the effect of an applewood extract and BHT on lipid oxidation in a model emulsion system, representing for the cosmetic industry, during 12 weeks of storage at 45°C to determine the effect in antioxidant activity. The oxidative stability was studied by measuring hydroperoxides, aldehydes and the fatty acid profile of the emulsions during storage. Prior to this, the apple wood extract was screened through *in vitro* antioxidant tests and polyphenol profiling via HPLC-DAD.

Results:

The HPLC-PDA analysis demonstrated that apple wood extract contains a significant amount of polyphenols, in the form of flavonoids. This explains why this extract also exhibits high antioxidant capacity measured via the *in vitro* tests. Additionally, a cosmetic formulation with 100 ppm polyphenols, derived from applewood extract, was successfully produced. Addition of applewood extract (100 ppm) and BHT (100 ppm) decreased the formation of hydroperoxides and malondialdehyde in the cosmetic formulations.

Conclusion:

The results of this study emphasize that applewood extract has the potential to act as a natural antioxidant and thus replace synthetic stabilizers. The results open ups a new market possibility for the re-use of applewood wastes as cosmetic products with antioxidant and preservative effect.

P 069 SANGGENON G, A DIELS-ALDER ADDUCT SCREENED FROM MORI CORTEX, ATTENUATED IRINOTECAN-INDUCED GASTROINTESTINAL TOXICITY IN MICE THROUGH SUPPRESSING SN-38 INTESTINAL ACCUMULATION VIA A DUAL INHIBITION MECHANISM

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Gut microbial β-glucuronidases (GUSs) inhibition is an emerging approach to manage disease and medication therapy[1]. Recent studies indicate that flavonoid compounds could be a promising source of microbial GUSs inhibitors[2]. However, the extreme structural and functional complexity of GUSs has posed big challenges to identifying GUSs involved in a specific reaction, hindering the discovery of potent specific inhibitor to meet varied clinical needs. To cope with the challenges, we employed a specific reaction guided broad-spectrum GUS inhibition strategy in the discovery of inhibitors for alleviating Irinotecan (IRT)-induced gastrointestinal toxicity. IRT is the first-line treatment of advanced colorectal cancer (CRC). But its dose intensification was limited by severe diarrhea resulted from regeneration of the toxic metabolite SN-38 by gut microbial GUSs[3,4]. Specifically, inhibitors were screened *in vitro* using a screening system consisting of human gut microbiota pool, representative *gus*-harboring bacterial isolates and GUS proteins selected based on their metabolic profiles towards an endogenous glucuronides pool and/or reported roles in CRC. Natural compounds of different chemical types were screened, among which flavonoid compounds, in particular the Diels-Alder (DA) adducts uniquely distributed in the plants of Morus genus, showed strong GUS inhibition activity towards the pool. Using activity-guided isolation with the pool combining virtual screening with *Eco*GUS from *Escherichia coli*, *Spas*GUS from *Staphylococcus pasteuri* and *Saga*GUS from *Streptococcus agalactiae*, we finally predicted GUS inhibition potentials of >40 DA compounds using MOE software and identified key structural factors for the protein-inhibitor interactions. Six DA compounds were isolated from Mori Cortex, all showing strong inhibition towards the GUS activity of the pool (73–94% at 100 μM), the isolates and the proteins. Sangennon G (SGG) which showed IC_{50} of 0.16–0.64 μM towards purified GUSs was further assessed in mice. SGG (0.5 mg/kg, p.o. twice daily) protected mice from severe diarrhea, high mortality and intestinal injury induced by IRT (50 mg/kg/day, i.p.), agreeing well with decreased GUS activity, increased intestinal SN-38 glucuronidation and diminished colonic SN-38 accumulation. It's interesting to note that SGG also effectively suppressed OATP2B1-mediated SN-38 uptake by Caco-2 cells (IC_{50} 4 μM). Amoxapine (2.50 mg/kg, p.o. twice daily), a potent *Eco*GUS inhibitor, was less effective in rescuing irinotecan-induced toxicity due to weaker GUS inhibition (the pool IC_{50} >100 μM) and nil effect on SN-38 intestinal uptake. In conclusion, our specific reaction guided broad-spectrum GUS inhibition strategy was demonstrated to be feasible to cope with the extreme complexity of gut microbial GUSs. The dual inhibition mechanism makes SGG superior to other known GUS inhibitors in alleviating IRT toxicity. [Supported by the Science and Technology Development fund of Macao SAR (0091/2021/A2, 0098/2019/A2), Shenzhen-Hong Kong-Macau Science and Technology Program Category C (SGDX20210823103805038), Guangdong Natural Science Fund (2019A1515012195), and University of Macau (MYRG2018-00091-ICMS-QRCM)].

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P 070 A RANDOMISED CONTROLLED TRIAL TO INVESTIGATE THE COGNITIVE, MOOD AND METABOLIC EFFECTS OF ACUTE OYSTER MUSHROOM INTERVENTION IN OLDER ADULTS (OYSACO)

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Background:

The *Pleurotus* oyster species is a common edible mushroom rich in ergothioneine, a bioactive compound with known neurocognitive benefits. However, no human clinical trial has specifically examined the effects of oyster mushrooms on cognitive function and mood in the immediate postprandial period.

Objectives:

Using a double-blind, randomised, cross-over design, the objectives of this study were to investigate whether ergothioneine-rich oyster mushrooms induce a) cognitive and mood benefits as well as b) improvements in the markers of metabolism and inflammation, in the acute post-prandial period (6 h post-consumption) in healthy older adults.

Methods:

Healthy adults (n = 33) aged 60–80 years old were asked to consume a noodle soup containing the equivalent of 0.5 (OM0.5), 1 (OM1) and 2 (OM2) servings of powdered *Pleurotus* oyster mushroom and a calorie-matched control soup (OM0), on four separate occasions with one-week washout between test days. Cognitive function and subjective mood were assessed at baseline (-1 h, prior to administration of the intervention) and then at 2 h, 4 h and 6 h post-consumption on each test day. The computerised battery included tasks assessing mood (Positive and Negative Affect Schedule, PANAS), episodic memory (Rey's Auditory Verbal Learning Task, RAVLT), executive function (Task Switching Task, TST), working memory (Corsi Blocks Task, CBT), and psychomotor function (Finger Tapping Task, FTT). Visual Analogue Scales were used to record palatability ratings immediately after consuming each intervention (0 h), and to monitor appetite throughout the day (-1 h, 0 h, 2 h, 4 h and 6 h postprandially). Finally, a serum blood sample was taken at the end of each test day (6 h) to examine metabolic and inflammatory markers.

Results:

All cognitive and mood data were analysed using Linear Mixed Modelling (LMM), with baseline included as a covariate, and Bonferroni-corrected pairwise comparisons. Mood data for positive affect and mental fatigue revealed significant main effects of time, with declining positive affect and increasing fatigue across the day. Subsequent pairwise comparisons revealed significant decline between 2 h and 6 h only after consuming the control (OM0). However, levels of positive mood and fatigue remained stable throughout the course of the day following consumption of oyster mushrooms (OM0.5, OM1, and OM2). Cognitive findings were mixed. TST data revealed a significant main effect of time and treatment*time interaction for both accuracy and reaction time. Pairwise comparisons revealed higher accuracy at 2 h for OM2, and at 4 h for OM0.5, compared to OM0. However, for OM0, accuracy scores significantly increased across the day, and mushroom benefits to accuracy performance were no longer apparent at 6 h. No reaction time benefits of oyster mushroom were observed compared to OM0. RAVLT delayed recognition data showed a treatment main effect and interaction, but pairwise comparisons between oyster mushroom conditions and the control were found to be non-significant over the 6 h period. No significant findings were observed for CBT, FTT, or blood markers.

Conclusion:

This study has shown a maintenance of positive affect and mental fatigue, up to 6 h post-consumption of ergothioneine-rich oyster mushrooms. Acute cognitive and metabolic benefits are less clear and may require a longer period of daily consumption to emerge.

P 071 ROLE OF SULFORAPHAN ON THE INFLAMMATORY IMMUNE RESPONSE

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Background:

Sulforaphane (SFN), an isothiocyanate of vegetable origin found in cruciferous, such as broccoli, presents potent antioxidant and immunomodulatory properties. These pleiotropic activities derive from their ability to influence multiple signaling pathways, highlighting the Nrf2/HO-1 pathway. Specifically, the induction of this signaling pathway in dendritic cells (DCs) produces their polarization towards a tolerogenic phenotype.

Objective:

The aim of this work was the study the immunomodulatory effect of the SFN in an inflammatory microenvironment on monocyte-derived dendric cells (moDCs).

Methods:

We characterized the immune and antiinflammatory response to SFN in moDC from healthy subjects (N=5). Lipopolysaccharides (LPS, 100 ng/mL) was added to induce the inflammatory environment cell. After that, immune and anti-inflammatory response in presence of SFN (10 and 20 µM) was analyzed. Thus, the SFN internalization and cytotoxicity by moDCs, moDCs maturation (HLA-DR, CD40, CD80, CD83, CD86) and T-lymphocytes proliferation and ROS assay were evaluated by flow cytometry.

Results:

Results demonstrated that SFN was up taken and processed by moDCs, exerting a low cytotoxic effect on them. Moreover, SFN significant reduced CD83 and CD80 maturation markers in moDCs and their ability to stimulate T-lymphocyte proliferation in a context of chronic inflammation. In addition, a decrease in the cytoplasmic levels of Nrf2 and pNrf2 expression with a significant increase in the HO-1 expression were observed moDCs, indicating the important role of the SFN in the prevention of inflammation.

Conclusion:

Our findings indicate that the use of this nutraceutical capable of inducing a decrease moDCs maturation state and an increase in the HO-1 enzyme opens a new perspective in the treatment of pathologies with an inflammatory and/or autoimmune component.

P 072 WHAT DO WE KNOW ABOUT CLIMATE CHANGE AND FOOD BIOACTIVES: THE CASE OF GLUCOSINOLATES

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The Brassicaceae family consists of numerous taxa, genera, and tribes, making it a diverse group of plants found worldwide, except Antarctica. This family holds significant economic, agronomic, and medicinal importance. At the nutritional level, in addition to vitamins (A, B1-2-6, C, E, K) and minerals (magnesium, iron, calcium), they also contain a relevant class of bioactive compounds, i.e. glucosinolates (GLS). GLS, through the conversion into their metabolic derivatives, can promote health benefits mainly related to their capacity to positively modulate important detoxification and inflammatory pathways. GLS bioactivity may be dependent on their food content, level of intake and bioavailability. Numerous factors seem to affect food bioactive concentrations, including GLS in Brassica vegetables, and climate change could represent a significant variable in this context. In fact, climate change poses challenges to dietary diversification and the promotion of agricultural sustainability to ensure an adequate supply of nutrients in the future.

To assess the extent of knowledge on the interaction between climate change-related factors and GLS content and type in foods, a systematic review has been conducted, also evaluating the potential implication for human nutrition and health.

Studies were collected from PubMed, Embase, and Scopus databases. Eligible studies investigated any effect or relation of climate change on the Brassicaceae family, specifically focusing on changes in GLS. Studies that combined interventions with other factors, such as insects, fertilization, UV light, or other treatments that could be confounding factors, were excluded unless individual experiments were conducted for each measured variable.

A total of 68 studies were analyzed, with most being completely randomized. The studies primarily focused on broccoli, oilseed rape, and kale as food sources, examining the effects of temperature, drought, and CO2 levels on GLS concentrations. Extreme temperature ranges were found to increase total GLS concentrations. Drought, waterlogging, elevated CO2 levels, and higher soil salt concentrations influenced the concentration and types of GLS in food, depending on the vegetable, crop variety and genetics. Altered glucosinolate content may affect the overall antioxidant, anti-inflammatory and protective capacity of cruciferous vegetables, potentially impacting their beneficial role. While glucosinolates offer health benefits it has been also reported that higher concentrations may have negative effects such as goitrogenicity, drug interference, digestive intolerance, and individual variabilities. Additionally, some plants with increased GLS may have a bitter or pungent taste, impacting food palatability and reducing consumption.

Overall the results obtained underline the adaptation of plants in response to environmental stresses, that can involve an overproduction of GLS in some conditions. However, the heterogeneity of data available does not allow to determine the magnitude and nature of changes. Thus, further research is needed to understand the full implications of climate-induced changes in glucosinolate content, their impact on future scenarios in terms of intake and benefits of these compounds.

Keywords:

Climate change; Glucosinolates; Isothyocyanates; Food content; Human health

P 073 BIOAVAILABILITY OF ORGANOSULFUR COMPOUNDS AFTER INGESTION OF BLACK ONION BY HEALTHY SUBJECTS

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Background:

Onion (*Allium cepa* L.) and the new derived product "black onion" are characterised by the presence of compounds with potential bioactivity, particularly organosulfur compounds (OSCs). Previous studies have related the regular consumption of organosulfur compounds to protective effects against several diseases such as some types of cancer, cardiovascular and neurodegenerative diseases, and metabolic syndrome. However, the health potential of the regular consumption of black onion, and thus of its phytochemical composition has not yet been evaluated.

Objective:

This study aims to monitor the excretion of OSCs in urine after an acute intake of black onion by healthy subjects.

Methods:

An intervention study was performed involving 12 volunteers (5 men and 7 women) aged between 22 and 49 years, with a body mass index between 19.6 and 26.8 kg/m^2. The identification and quantification of organosulfur compounds in urine was carried out by ultra-high performance liquid chromatography coupled to high performance mass spectrometry (UHPLC-HRMS).

Results:

A total of thirty-one OSCs were detected in urine after the acute ingestion of black onion, with *S*-methyl-L-cysteine sulfoxide (methiin) (13628 ± 3871 nmol), isoalliin (12356 ± 4706 nmol) and *S*-propyl-L-cysteine (deoxypropiin) (3135 ± 704 nmol) being the main metabolites excreted in urine. Moreover, it has been determined potential *N*-acetylated metabolites of the major OSCs present in black onion, namely *N*-acetyl-*S*-(1-propenyl)-L-cysteine sulfoxide (NAS1PCS), *N*-acetyl-*S*-(1-propenyl)-L-cysteine (NAS1PC). The *N*-acetylation reaction takes place in the kidney and liver, and metabolization pathways have been proposed to explain the urine excretion of some of these organosulfur compounds. The main excretion of organosulfur compounds was produced between 8 and 24 h after the black onion consumption, comprising 52% of the total amount excreted, highlighting that the absorption of these compounds mainly occurs at intestinal level. Nevertheless, a significant percentage of organosulfur compounds can also be absorbed at the gastric level since 22% of the γ-glutamyl-*S*-alk(en)yl-L-cysteine derivatives and 32% of the *S*-alk(en)yl-L-cysteine derivatives were excreted from 0 to 4 h.

Conclusion:

The identification of these organosulfur compounds as urinary metabolites after black onion ingestion has been described for the first time in this study and provides the basis for future approaches.

P 074 CHEMICAL COMPOSITION AND QUALITY PARAMETERS OF BUTTERNUT PUMPKIN SEED OIL

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Nowadays, the implementation of a circular economy for the valorization of agri-food by-products is one of the main challenges facing the agri-food industry. The pumpkin seed is a by-product obtained during the cutting and preparation of the edible part of the pumpkin as a minimally processed product. These seeds can be used to extract the fat through different procedures, obtaining a vegetable oil rich in PUFA, carotenoids, phytosterols and phenolic compounds, with several beneficial biological activities.

The aim of this work was to determine the chemical composition, quality parameters and the content of carotenoids in oil from seeds of butternut pumpkin (*Cucurbita moschata*), as well as to evaluate its stability after applying thermal treatment.

Oil was extracted from butternut pumpkin seeds by extrusion, after drying the seeds in an oven at 120 ºC for 2 h. Proximate composition (moisture, protein, carbohydrates and total fat), peroxides and acidity values were analyzed according to the AOAC method. Color parameters were registered using a Minolta refractometer, total carotenoids were quantified by a spectrophotometric method and individual carotenoids by HPLC-DAD analysis, and the fatty acid profile was determined by GLC. The oil was subjected to a heat treatment at 100ºC for three and a half hours, aliquots were taken to analyze the evolution of the quality parameters.

The oil was mainly composed of total fat (74 %), followed by protein (15.7 %), showing an intense dark orange color (hue angle 42º) due to the high content of carotenoids (5–6 mg/100 g). Related to the fatty acids, the oil contained a 72 % of PUFA, 24 % of MUFA y 4 % of SAFA. The main carotenoids were β-carotene, around 50 %, followed by α-carotene (30 %) and lycopene (5 %). During the thermal treatment, a slight tendency was observed in the quality parameters, with a decrease in carotenoids in a 20 % and increase in acidity and peroxide index. However, no significant differences were observed, and the fatty acids profile remained unchanged.

Due to the extraction process, pumpkin seed oil can be considered safe for consumption, providing several bioactive compounds particularly carotenoids, so has a great potential for use as a functional oil. Further studies are needed to determine its specific applications and the desirable daily consumption.

P 075 CAROTENOID AND ANTIMICROBIAL COMPOUND PRODUCTION FROM THE GROWTH OF THE MARINE FUNGUS *Asteromyces cruciatus*

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There has been great interest in discovering and producing compounds with bioactive properties for the cosmetic, food, and biomedical industries, as well as focusing on economical and environmentally friendly production.

This research mainly focuses on the use of unconventional and less exploited raw materials, such as macroalgae, lignocellulosic materials, and/or agricultural residues. Some researches are based on the production of secondary metabolites and proteins through fermentative process of macroalgae for the cultivation of microorganisms, since in these cases it is possible to control factors that improve the growth of microorganisms and the production of compounds. One type of secondary metabolites is carotenoids, which have been used in the industry to improve the coloring of products and take advantage of their bioactive properties such as antioxidants and anti-inflammatory agents. Recently, their production from natural sources has gained interest since the synthetic pigments have caused adverse health effects. In addition, thanks to the development of metabolomics, its possible to analyze and compare produced metabolites by a microorganism under specific conditions.

On the other hand, *Asteromyces cruciatus* is an underexploited marine-derived fungus, mainly cultivated on a defined medium, in which secondary metabolites, some of them with antimicrobial activity, have been discovered.

In this work, we will analyze whether the variation in culture factors (such as nutrients, pH and salinity) and assimilation of the macroalgae *Durvillaea spp., M. pyrifera, G. skottsbergii,* and *A. chilensis* affect fungi growth and the production of secondary metabolites, such as carotenoids. For these experiments, two methodologies for mycelium carotenoid extraction were tested, one called Heat-Acid and the other called Physical Disruption, in order to evaluate if these two methodologies had effects in the concentration of total produced carotenoids and also the type of carotenoid extracted. Moreover, in this investigation, the total metabolites were extracted from the mycelium and a preliminary analysis was performed to determine if this extract had antibacterial activity against *Escherichia coli*.

Preliminary results have shown that increasing NaCl concentration (1 to 3% NaCl) favors fungal growth with a 11 g/L of DW biomass and the total carotenoid concentration (50 mg/L), and increasing pH (5 to 8), and decreasing yeast extract concentration (6 to 4 g/L) negatively affect pigment production. Both methodologies showed similar concentrations of total carotenoid content but had differences in the HPLC chromatograms. For this reason, Physical Disruption is the better methodology because in its chromatograms there are peaks that were not obtained with the other methodology. Finally, *A. cruciatus* cultured with the macroalga *M. pyrifera* produces metabolites with antimicrobial activity against *Escherichia coli*. Therefore, it is necessary their further characterization

The next step of this research is to continue with the analysis of the secondary metabolites of *A. cruciatus* cultured with different macroalgae, their identification, and the analysis of the bioactive properties such as antioxidant, neuroprotective, and antimicrobial activity with another microorganism to produce a bio-composite with an ecological fermentation process.

P 076 COMPARISON OF TRADITIONAL VS THE BIOSOLVENTS ETHYL LACTATE AND 2-METHYLTETRAHYDROFURAN FOR THE EXTRACTION OF *Dunaliella bardawil* CAROTENOIDS

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Background:

Carotenoids have long been recognized for their natural colorant properties and provitamin A content, making them a significant factor in food science and nutrition. Recently, there has been an increased interest in these compounds due to mounting scientific evidence of their potential health benefits, including antioxidant and anti-inflammatory properties. The food industry is placing a greater emphasis on sustainable technologies, with ultrasound-assisted extraction (UAE) gaining popularity due to its ability to enhance carotenoid extraction while reducing solvent consumption and extraction time. The use of green solvents, which are non-toxic, recyclable, and biodegradable, is also in demand. Ethyl lactate has been demonstrated to be a potent extraction solvent for various compounds in previous studies. In addition, 2-methyltetrahydrofuran (2-MeOx) is a promising green solvent that requires further investigation.

Objective:

The aim of this study was to compare the total carotenoids extracted through solvents authorized for used food and emerging green solvents.

Methods:

The freeze-dried, fresh, and encapsulated matrices used for this study were *Dunaliella bardawil* UTEX 2538. The UAE of carotenoids from microalgae was carried out at 30% amplitude, 20 kHz frequency, and for 2 minutes. Five different solvents were tested: ethanol, methanol, ethyl lactate, 2-MeOx, and dimethylsulfoxide (DMSO). The best solvent and conditions were selected and the results were compared to emerging sustainable solvents. The effect of pre-treatment of micro-mill (30 Hz, 5 min) was also studied.

Results:

The application of the micro-mill in *D. bardawil* did not result in statistically significant effects (p>0.01) on individual and total carotenoid content (TCC) in all samples evaluated. Previous investigations conducted within our research group demonstrated that the application of the mill significantly increased the release of carotenoids in freeze-dried and encapsulated *C. sorokiniana*. These findings may be attributed to the rigid cell wall of *C. sorokiniana*, while *D. bardawil* has no cell wall, and hence, ultrasound treatment is sufficient to enhance carotenoid extraction. In terms of solvent selection, ethanol, and 2-MeOx exhibited the highest efficacy in recovering of lutein, β-carotene, 9-*cis*-anteraxanthin, α-carotene, 9-*cis*-β-carotene, and zeaxanthin, which were the primary carotenoids found in the sample.

With regards to TCC (Table 1), the solvent ethanol demonstrated the highest efficacy in the freeze-dried sample, followed by methanol, 2-MeOx, and ethyl lactate, with no significant differences observed between the latter three solvents. In the fresh sample, the most efficient solvents were 2-MeOX, methanol, and ethanol, with no significant differences noted between them. Finally, in the encapsulated matrix, 2-MeOx and ethanol were identified as the most effective solvents for TCC, with no statistically significant differences observed between them.

Table 1: TCC (mg/g) in *D. bardawil.*

	Freeze-dried	Fresh	Encapsulated
Ethanol	13.63 ± 0.60 ^{C}	1.87 ± 0.04 ^{B}	0.39 ± 0.01 ^{C}
Methanol	11.62 ± 0.71 ^{B}	1.96 ± 0.05 ^{B}	0.30 ± 0.02 ^{B}
Ethyl lactate	10.74 ± 0.70 ^{B}	1.64 ± 0.12 ^{A}	0.15 ± 0.00 ^{A}
2-MeOx	10.88 ⊥ 0.21 ^{B}	2.04 ⊥ 0.05 ^{B}	0.42 ⊥ 0.02 ^{C}
DMSO	6.83 ± 0.47 ^{A}	1.50 ± 0.09 ^{A}	0.17 ± 0.00 ^{A}

Conclusion:

2-MeOx is an excellent novel emerging green solvent as an alternative to other organic solvents for the extraction of carotenoids from *D. bardawil*.

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P 077 ISOLATION OF CARROT CHROMOPLASTS AND EVALUATION OF THEIR CAROTENOID CONTENTS AND BIOACCESSIBILITY

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Background:

Chromoplasts are plastids key for the biosynthesis and accumulation of carotenoids in plant cells. Additionally, chromoplasts actively participate in different metabolic processes during fruit ripening. They derive from chloroplasts that lose chlorophyll pigments and favour an increased biosynthesis of carotenoids. Although it is well-known that carotenoids can be accumulated in different ways leading to different chromoplasts (globular, crystalline, fibrous, and membranous), little is known about the carotenoid profile and bioaccessibility of different chromoplast fractions.

Objectives:

The purpose of this study was to isolate chromoplast fractions from carrot and evaluate differences in carotenoid levels and bioaccessibilities among them.

Methods:

Fresh carrots purchased from a local store were studied. Chromoplast fractions were isolated following the methodology proposed by Angaman et al. (2012). The fractions obtained were recovered and carotenoid extracts were obtained, followed by HPLC analysis following the protocol validated by Stinco et al. (2019). For bioaccessibility assessments the standardised static *in vitro* static digestion protocol described by Brodkorb et al. (2019) was followed.

Results:

Three bands of chromoplasts corresponding to 15%, 30% and 40% sucrose were obtained in the gradient. The distribution of carotenoids in these bands was similar. The colourless carotenoids phytoene (PT) and phytofluene (PF) were identified in all bands, as well as ζ-carotene (ZCAR), lutein (LUT), and the provitamins A α-carotene (ACAR) and β-carotene (BCAR), which were the most abundant accounting for approximately 40% of the carotenoids present in the bands.

Considering the micellar fractions obtained after the digestions, for all bands, the carotenoids found in the highest proportion were the colourless ones (41.7% PT and 28.4% PF). The provitamin carotenoids and ZCAR were similarly distributed in the three bands, with the concentration of ZCAR being approximately 19%, ACAR almost 8%, and BCAR only 0.2%. LUT, on the other hand, showed an uneven distribution ranging from 3.2% in the 15% band,, 4.1% in the 40% band and 0.5% in the 40% band. Regarding the percentage of bioaccessibility, it was observed that the 40% band showed the highest bioaccessibility, with an approximately three-fold higher content in all the carotenoids identified, except for LUT. The highest bioaccessibility of LUT was observed in the 30% sucrose band, with a bioaccessibility twice as high as that in the other bands.

Conclusion:

Three distinct chromoplast-containing bands corresponding to 15%, 30% and 40% of the sucrose gradien were isolated. Their carotenoid profile was similar qualitatively and quantitatively. For each band, the distribution of carotenoids in the micellar fractions obtained upon *in vitro* digestions was similar for all carotenoids except for lutein. The bioaccessibility decreased in the order PT>ZCAR>PF>LUT>ACAR>BCAR.

P 078 EVALUATION OF THE EFFECT OF DIFFERENT COOKING METHODS ON THE LEVELS AND POTENTIAL BIOAVAILABILITY OF CHERRY TOMATO CAROTENOIDS

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Background:

The various culinary techniques that we commonly use cause changes in the physicochemical properties of foods. Most of these methods require the application of heat and can cause losses of nutrients and other food components. However, it can also improve texture and/or flavour, as well as the bioavailability of certain compounds such as carotenoids. Many studies indicate that thermal treatments can cause modifications such as the breaking of cell walls and membranes in the matrix, which promotes the release of these compounds. The processing time and heating conditions can determine the level of modification of the cell structures and therefore the changes in the carotenoid levels and potential bioavailability compared to the raw matrix. This study evaluated the impact of different cooking conditions on the contents and bioaccessibility of cherry tomato carotenoids present in tomatoes in the context of contributing to the promotion of healthier cooking methodologies.

Objectives:

The goal of this study was to evaluate the effect of different cooking conditions on the contents and bioaccesibilities of cherry tomato carotenoids.

Methods:

Approximately 100 g of Cherry Rama Cat.II Cal. A tomatoes were used for each type of treatment. The cooking methods used were: air fryer at 180, 190, and 200 °C for 2, 6, and 10 minutes. Conventional oven at 180, 190, and 200 °C for 5, 12.5 and 20 minutes. Microwaving at 420, 560, and 800 W for 30, 60 and 90 seconds. Cooking in water at temperatures 80, 90, and 100 °C for 5, 7.5, and 10 minutes. For carotenoid extraction and analysis, the method validated by Stinco et al. (2019) was followed and for bioaccessibility assessment, the standardized *in vitro* static digestion protocol described by Brodkorb et al. (2019) was followed.

Results:

The results obtained showed that, as expected, the different cooking methods produced in some cases a decrease in carotenoid concentration compared to the uncooked matrix (control), but these were not statistically significant. In general, longer cooking times resulted in a greater decrease in carotenoid concentration compared to the control in the undigested samples. The cooking treatments that produced the greatest increase in carotenoid bioaccessible content (CBC) compared to the control were air frying at low power for 6 minutes (that produced an increase of 50%) and baking for 20 minutes (that produced an average increase of 30%).

Conclusion:

As expected, the cooking conditions applied to the samples produced changes in the concentration of carotenoids compared to the control. More importantly, it was observed that the cooking of cherry tomatoes led in some cases to noticeable significant increases in the CBCs, that is, the amount of carotenoids that is incorporated in mixed micelles from a food serving and can be considered as potentially absorbable.

P 079 ELECTROSTATIC SPRAY DRYING: AN INNOVATIVE PROCESS TO ENCAPSULATE ENZYME

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Bioactive proteins such as enzymes show a growing interest in the industry. However, stabilizing over time while conserving its properties remains a challenge as it is easily degraded with temperature. Nowadays, two drying processes are mostly used as reference: Spray-Drying (SD) and Freeze-Drying (FD). On one hand, SD has damaging effects on bioactive molecules by working at high temperatures, but it is a well-established process for obtaining high production rates. On the other hand, freeze-drying can dry sensible molecules and preserve their bioactivities, but the technology is highly time and energy consuming. A new technology is emerging as an alternative process: Electrostatic Spray Drying (ESD). ESD is working at lower temperature than SD thanks to the use of an additional electrostatic field. This field allows a faster migration of the solvent to droplet's surface and creates a thin layer of water available for drying. In this study, β-galatosidase, was used as reference enzyme to compare the influence of matrices and drying processes. This enzyme can break down lactose into glucose and galactose which has interest for lactose intolerance disorder.

The aim of the study was to compare matrices and processes for drying β-galatosidase. Enzyme activity after encapsulation was compared regarding the process and formulations. Two types of carriers were chosen: skim milk and shellac as matrices to protect the enzyme during the drying. The residual enzyme activity was analyzed based on lactose hydrolysis and quantified. Lactose degradation was linearized using an order 1 model. A life cycle analysis (LCA) was also performed to compare energy consumption and carbon footprint of processes. 16 indicators were used to compare the impact of producing 1 kg of powder expressed in kg CO_2.

Results show that it is possible to preserve the enzyme from degradation by using shellac and skim milk. However, the enzyme activity drops to 0 after 12 months storage at 21 °C. when no encapsulation is conducted or by using SD with shellac. The highest enzyme preservation was obtained with ESD and shellac (-0.8 h^{-1} at 0 month and-0.5 h^{-1} at 12 months) compared to freeze-drying (-0.1 h^{-1} for 0 and 12 months). Similar trends are observed with skim milk as protective matrix where SD shows the lowest enzyme activity (-0.3 h^{-1}). ESD and FD powders have comparable lactose degradation rates which are stable for 12 months (-0.6 h^{-1}). LCA study highlights that FD process has three times higher electricity consumption compared to ESD. Moreover, the climate impact of skim milk is at least ten times superior compared to shellac.

To conclude, formulations and drying processes show a high influence on the enzyme activity preservation of β-galactosidase. ESD seems a promising technology for encapsulating over time as it shows higher lactose degradation rate with shellac. LCA study indicates that shellac has lower impact on climate change while ESD and SD processes show lower energy consumption.

P 080 COD MEAT PROTEIN HYDROLYSATES WITH POTENTIAL SIGNIFICANCE IN THE PREVENTION OF CIVILIZATION DISEASES – BIOINFORMATIC RESEARCH

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Bioactive peptides, the source of which is food, can be beneficial in the prevention of civilization diseases. In the presented research, the potential usefulness of Atlantic cod (*Gadus morhua*) proteins as a source of antihypertensive, antidiabetic and antioxidant peptides, which were released in silico by the enzymes: pepsin, trypsin and chymotrypsin, was determined.

Protein sequences (421) were obtained from the UniProtKB database, then the Clustal Omega program was used, thanks to which amino acid sequences similar in no more than 80% were selected for further research (78). In BIOPEP-UWM, profiles of potential biological activity of proteins and the frequency of occurrence of peptides with specific activities were determined, and gastric and gastrointestinal hydrolysis was performed in silico and the frequency of release of specific biopeptides was determined. Potentially released biopeptides were analyzed for their toxicity (ToxinPred), bioactivity potential (PeptideRanker) and absorption in the human digestive system (ADMETlab).

During the research, it was proved that cod muscle proteins can be a source of peptides with the activity of ACE inhibitors, DPP-IV inhibitors, DPP-III inhibitors, renin inhibitors, and alpha-glucosidase inhibitors, as well as antioxidant peptides. It has also been proven that peptides with such activities can be released during simulated protein digestion. All potentially released biopeptides with activities relevant to the prevention of civilization diseases were defined as non-toxic, and the vast majority consisted of 2 or 3 amino acids. The results showed that the highest potential frequency of occurrence and potential release frequency have peptides with DPP-IV inhibitory and ACE inhibitory activities.

To sum up, it can be assumed that the muscle proteins of Atlantic cod may be useful in the prevention of civilization diseases, such as hypertension and diabetes.

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P 081 YOGHURT ENRICHED WITH MILK SERUM PROTEINS MIXED WITH BUTTERMILK CONCENTRATE AS A SOURCE OF BIOLOGICALLY ACTIVE PEPTIDES RELEASED VIA DIGESTION

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Milk is a rich source of essential nutrients and also bioactive peptides. Dairy powders, depending on their composition, are increasingly used in the food processing industry, including dairy products such as ice-cream, yoghurt, and cheese. Biopeptides from milk proteins, can be generated by gastrointestinal digestion after consumption. Yoghurts enriched with milk serum proteins mixed with buttermilk concentrate (MSPB) with and without lactose were *in vitro* digested and their bioactivity were analyzed.

The research material consisted of the following dairy beverages: JK 6- control yoghurt, JMSPB-P6- yoghurt with the addition of MSPB, JMSPB-PL6 – lactose-free yoghurt with the addition of MSPB. The Infogest digestion method consisted of the following steps: "oral", "stomach" – 1 hour, pH=3, "duodenal" - 1 hour, pH=7.0. Hydrolysates were analysed for their enzyme inhibitory (ACE, DPP-IV, α-glucosidase, and lipase) and antioxidant activities. The hydrolysates were used in a screening for bioactive peptides by RP-HPLC-ESI-MS/MS method.

The digests of yoghurts showed ACE, DPP-IV, α-glucosidase, and lipase inhibitory activity, as well as antioxidant activity. The difference between control yoghurt, JMSPB-P6 and JMSPB-PL6 samples were observed. Among all yoghurt samples subjected to simulated digestion (phase D), the most active towards ACE inhibition was JMSPB-P6 (IC_{50}=1.556 mg/ml). Comparing the DPP-IV inhibitory activity of samples before and after digestion, the most active was JMSPB-PL6. The highest α-glucosidase inhibitory activity was shown by digest of JMSPB-P6 yogurt (IC_{50}=0.0530 mg/ml). The highest lipase inhibiting ability was detected for JMSPB-P6 (IC_{50}=0.409 mg/ml). Regardless of the test used to measure antioxidant activity- all samples were characterized by the antioxidant activity. In the case of the ABTS test, it was shown that JK6 digest was the hydrolyzate with the highest antioxidant activity (IC_{50}=3.911 mg/ml). The DPPH test showed that JMSPB-PL6 digest had the highest antioxidant activity (IC_{50} mg/ml). Of the total 52 identified, 33 peptides have had IC_{50} values. These are mainly ACE and DPP IV inhibitors. Examples of identified peptides include: the ACE inhibitors (eg. IPA, IR), DPP-IV inhibitors (eg. IPA, IR, PW) and antioxidant fragments (eg. IR, PW).

Yoghurts enriched with MSPB can be considered as an interesting sources of peptides with biological activity, including inhibitors of enzymes, as well as antioxidant peptides that are released after digestion.

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P 082 CHARACTERISTICS OF PEPTIDES POTENTIALLY RELEASED DURING DIGESTION OF CHICKPEAS PROTEINS – BIOINFORMATIC RESEARCH

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Nowadays, society is increasingly struggling with lifestyle diseases that can significantly reduce the quality and length of life. Civilization diseases include: cancer, type II diabetes, problems with the circulatory system, i.e. hypertension, atherosclerosis, heart attacks, etc.

Chickpeas are considered to be one of the first crops cultivated by humans for consumption and processing. The most valued species of chickpea is common chickpea (*Cicer arietinum* L.). It owes its popularity to the fact that it is rich in nutrients such as fiber, B vitamins, minerals and easily digestible protein. A positive feature of chickpeas is the high content of proteins, which ranges from 19–25 g per 100 g. Chickpeas are considered to be one of the richest sources of vegetable proteins.

The aim of the study was to characterize potentially bioactive peptides released during simulated digestion of chickpea (*Cicer arietinum* L.) proteins using bioinformatics methods. In this study, attention was focused on peptides with significant activity in the prevention of civilization diseases. Peptides with antihypertensive activity – angiotensin I-converting enzymes (ACE) inhibitors, antidiabetic activity – dipeptidyl peptidase IV (DPP-IV) and antioxidant peptides were searched for.

On the basis of computer research conducted using databases and programs available on-line (UniProtKB, Clustal Omega, BIOPEP-UWM, ToxinPred, PeptideRanker), information on biopeptides encrypted in amino acid sequences of chickpea grain proteins was obtained. Thanks to these tools, profiles of potential biological activities of proteins and frequencies of fragments with ACE inhibitory, DPP-IV inhibitory and antioxidant activity were established. The analyzed chickpea proteins, especially globulins and glutelins, contained biopeptides with selected activities in their sequence.

Simulated *in silico* gastric and gastrointestinal digestions were then performed. The study indicated the possibility of releasing peptide inhibitors of enzymes important in the prevention of civilization diseases after hydrolysis with pepsin, trypsin and chymotrypsin. Chickpea proteins were a rich source of peptidic DPP-IV inhibitors and, to a lesser extent, ACE inhibitors. The biopeptides released from chickpea proteins were mainly dipeptides with hydrophobic properties and no toxicity.

Biopeptides potentially released from chickpea proteins may be useful in the prevention of civilisation diseases.

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P 083 POTENTIAL OF CRUSTACEAN SHELL WASTE FOR RELEASE OF PROTEIN-DERIVED PEPTIDES FOR HEART HEALTH (CRUSHH)

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Aim of this project was to explore the potential of crustacean shell waste for targeted health applications. With the rise in obesity and chronic metabolic disease in the population, posing a huge burden to the health system, there is clearly a scope for development of functional products that could contribute to prevent and/or alleviate some of these conditions. Crustacean shell is a by-product of the seafood processing industries which is available in large quantities yet at present not fully utilized. The current project was targeting the waste hydrolysate liquor stream resulting from a previously developed deproteinization process. The objectives included targeted hydrolysis using different enzyme conditions, fractionation and characterization of small molecular weight peptides in hydrolysed fractions as well as application of *in vitro* functional assays to determine biological activities. The results demonstrate the time-dependent increase in hydrolysis progress over the first 6 hours, and concomitant increase in antioxidant activity. Biological activity data indicate the presence of bioactive protein fragments in particular in the small molecular weight fractions (<3 kDa) which has been confirmed via independent methods (SDS-PAGE, HPLC). A significant increase in specific biological activity i.e. ACE-I inhibition as an indicator of blood-pressure lowering properties could not be clearly demonstrated under the current conditions used. Further work is therefore advised to optimise the enzyme-specific release of bioactive peptides for precise target applications, against the industrial process requirements; and with the ultimate aim to deliver innovative solutions that contribute to improve sustainability, resilience and productivity in the seafood sector.

P 084 FOOD-INDUSTRY BY-PRODUCT HYDROLYSATES AS A POTENTIAL FUNCTIONAL INGREDIENT FOR MANAGING ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is one of the most important neurological dysfunctions, representing 60- 70% of dementia cases. Its pathophysiology is characterized by the accumulation of ß-amyloid peptides and the hyperphosphorylation of Tau proteins in the brain. The enzyme prolyl-Oligopeptidase (POP) is thought to process the amyloid precursor protein, generating the ß-amyloid peptides. Ageing is the main risk factor for the development of the AD, and its prevention is crucial in the increasingly ageing population. In this sense, bioactive peptides are gaining prominence as nutraceuticals or functional ingredients, with agri-food industry by-products being an important source. Thus, the objective of this study was to obtain protein hydrolysates derived from a food-industry by-product, with neuroprotective properties. The food-industry by-product was milled, mixed with water (0.25 g/mL), and boiled for 1 h. Then, the mixture was centrifuged and the supernatant was used to prepare the protein hydrolysates. Sixteen hydrolysates were obtained under different hydrolysis conditions (4 enzymes at 2 concentrations of enzyme/substrate and 2 different hydrolysis times). The *in vitro* POP inhibitory activity of the hydrolysates was tested. The results showed that the majority of the hydrolysates (diluted 1/10) presented an inhibition of POP higher than 50 %. The most active hydrolysates were H7 and H9, which inhibited POP by 73.9 and 76.4 %, respectively. The CL4176 strain of *Caenorhabditis elegans* was used to evaluate the *in vivo* effectiveness of both hydrolysates (0.5, 1 and 5 mg/mL), as this strain develops paralysis due to the accumulation of ß-amyloid peptide in its muscular cells after heat induction. The percentage of paralyzed worms was measured every 2 h for the next 12 h after inducing paralysis. No differences in % of paralyzed worms were observed for any concentration of H9, either 0.5 mg/mL of H7. Conversely, 1 and 5 mg/mL of H7 hydrolysate were able to reduce the % of paralysis during the different timepoints since the 2 h after the induction in a range of 7- 28 % and 20- 33 %, respectively. Also, when this two concentrations of H7 were compared with the non-treated group at the final timepoint, significant differences were found (17 and 20 % less of paralyzed worms, respectively). The original protein solution without any hydrolysis was also studied as a negative control at 5 mg/mL. This control did not show any effect on worm paralysis, corroborating that the effect was a result of the hydrolysis and not from the native protein. These results suggest that H7 hydrolysate could be a good candidate as a nutraceutical or functional ingredient for AD prevention, although further studies are needed. Finally, this study highlights the potential of food industry by-products as a source of bioactive peptides, adding value to them, and contributing to the circular economy and sustainability of the sector.

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P 086 AGRI-FOOD BY-PRODUCT HYDROLYSATES ABLE TO INHIBIT ACE AND MODULATE GUT MICROBIAL POPULATIONS *IN VITRO*: INVOLVEMENT OF GASTROINTESTINAL DIGESTION AND ANTIHYPERTENSIVE EFFECTS

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The involvement of gut microbiota in the development of non-communicable diseases, including hypertension, has been established. However, the relationship and underlying mechanisms remain largely unknown. Given the high and increasing global incidence of this chronic disease, new preventive methods are necessary. One potential alternative is the development of natural compounds, particularly bioactive peptides, with the ability to inhibit the angiotensin-converting enzyme (ACE). The objective of this study was to assess the capacity of various protein hydrolysates to modulate *in vitro* microbial populations and angiotensin-converting enzyme inhibitory (ACEi) activity, taking into account the impact of gastrointestinal digestion on their bioactivity. Nineteen hydrolysates were derived from three food industry byproducts using different food-grade enzymes and hydrolysis conditions. The hydrolysates underwent *in vitro* simulation of gastrointestinal digestion using the Infogest method. ACEi activity was measured in the hydrolysates before and after gastrointestinal digestion. Both sets of samples were then subjected to 24 h fermentation with fecal human microbiota, and the bacterial composition was analyzed using qPCR. The antihypertensive effects of the three most promising hydrolysates were evaluated in spontaneously hypertensive rats following acute administration (55 mg/kg). All the hydrolysates demonstrated high ACEi activity (>90%), which remained unaffected by *in vitro* gastrointestinal digestion. Moreover, the hydrolysates exhibited differential abilities to modulate microbial populations, and gastrointestinal digestion altered this modulation in most cases, resulting in either an increase or decrease depending on the population analyzed. Three hydrolysates were selected based on their bioactivity for evaluation of their antihypertensive effects. Notably, one of these hydrolysates showed significant reduction in systolic blood pressure in hypertensive animals, particularly within the first 8 h post-administration.

P 087 BIOCHEMICAL CHARACTERIZATION OF SHEEP MILK YOALP® AND COMPARISON WITH SHEEP FERMENTED MILK OBTAINED WITH COMMERCIAL STARTER CULTURES

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Background:

Fermented milks are a source of bioactive peptides with different potential benefits on human health (mainly antioxidant, antihypertensive and hypoglycemic effects) and may be considered as functional foods. Furthermore, fermented milk made with sheep milk has higher nutritional value and higher concentrations of proteins, fats, minerals, and vitamins, if compared to milk of other species.

Objectives:

Aim of this study was to perform a biochemical characterization and a comparison between sheep fermented milk (SFM) made with commercial starter cultures and sheep milk YoAlp®, a fermented milk obtained using local strains of lactic acid bacteria (LABs) (Streptococcus thermophilus and Lactobacillus delbrueckii) which have been isolated during years in Aosta Valley mountain dairy cattle farm.

Methods:

Sheep milk (Sarda and Lacaune breeds) has been collected from "Azienda Agricola Morzenti" - Aymavilles (AO, Italy) and immediately analyzed. Biochemical characterization, by a proteomic approach, GC/MS and microtiter plate assay methods, have been conducted on fermented milks to evaluate their peptide, fatty acid and aromatic profile, and to assess potential health promoting effects.

Results:

The comparison between SFM and YoAlp® peptide profile shows a higher number of peptides using autochthonous starter cultures (52) than commercial starter cultures (35). Among these peptides 20,78% and 29,87%, respectively, are supposed to be potentially bioactive (DPP- dipeptidyl peptidase-IV- inhibitor, ACE- angiotensin converting enzyme – inhibitor and antioxidant).

In both products, the fatty acids profile was similar to that of origin sheep milk; concerning aromatic profile, YoAlp® shows yogurt typical aromatic assets, similar to the commercial one.

As far as bioactivity is concerned, ACE inhibitor activity is high for both samples. Similar values, as expected by peptide profile analysis, have been obtained using commercial and autochthonous starters. Even in the case of antioxidant capacity, peptide profile bioactivity prediction has been confirmed by the assay showing a DPPH inhibition higher for SFM than for YoAlp®. Finally, in contrast with peptide profile bioactivity assumption, DPP-IV inhibition is higher using Aosta Valley lactic acid bacteria if compared to commercial starter cultures.

Conclusion:

Local strains of lactic acid bacteria from Aosta Valley seems to work as well as the commercial, preserving biodiversity and typicity. However, further analyses are needed to identify peptides generated by autochthonous LABs in order to understand microbial proteolytic activities and to investigate gastric digestion resistance of bioactive peptides which can have positive effects on human health.

This study was part of TYPICALP, a project cofounded by EU, FESR, Italian Government, Helvetic Confederation and Swiss Cantons through Interreg V-A Italy Swiss cooperation program.

P 088 IMMUNOMODULATORY EFFECTS OF BIOAVAILABLE OLIGOPEPTIDES FROM HEMPSEED PROTEIN HYDROLYSATES VIA CACO-2/PRIMARY HUMAN MONOCYTES CO-CULTURE MODEL

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Background:

The incorporation of alternative proteins in human diets is quickly rising as a result of their health-promoting effects and environmental-friendly nature. Hemp (*Cannabis sativa* L.) seeds are exceptionally nutritious and rich in healthy lipids, good quality protein, and several minerals. Hemp protein hydrolysates (HPHs), obtained by food-grade enzymatic hydrolysis, are mixture of peptides that prior to reaching tissues and playing their biological function, at least being absorbed, and metabolized by the intestinal barrier. These bioavailable peptides that enter systemic circulation could be responsible of modulating several immunological processes.

Objectives:

The study aims to screen and identify high bioavailable and immunomodulatory HPHs-derived oligopeptides via Caco-2/primary human monocytes co-culture model.

Methods:

Hemp protein isolate, prepared from hemp defatted flour by alkaline solubilization/acid precipitation, was subjected to extensive food-grade enzymatic hydrolysis using Alcalase for 20 min (HPH20A) and using Alcalase for 60 min followed by Flavourzyme for 15 min (HPH60A15F). Caco-2 cells were cultured in 12-well cell culture inserts with fresh medium containing the HPH20A or HPH60A15F at 1 mg/mL in the apical chamber. After 4 hours, the content in the basolateral side was recovered in PBS (bioHPH20A and bioHPH60A15F). The peptidome of four HPHs was analysed using a nanoElute nanoflow ultrahigh-pressure LC system coupled to a timsTOF Pro 2 mass spectrometer, equipped with a CaptiveSpray nanoelectrospray ion source. To study the immunomodulatory effects of bioHPHs, in the basolateral side, LPS (1 µg/mL)-activated primary human monocytes were cultured and then the RNA was isolated to performed RT-qPCR. Furthermore, bioavailable peptides were assessed by *in silico* tools to hypothesize those that could be responsible of the bioactivity reported for the bioHPHs.

Results:

The bioavailable peptides contained in HPH20A and HPH60A15F were identified from the collected fraction from the transwell system employing Caco-2 cell culture as absorption model. Furthermore, 20 unique peptides corresponding to the most abundant <1000 Da peptides from that bioavailable fraction were assessed by *in silico* tools to hypothesize those that could be responsible of the bioactivity reported for the HPHs. From the identified peptides, based on the molecular features and the predictions, the peptides KNAIYTPH, EERPGHF, and KNGMMAPH, are proposed to be highly contributing to the biological activity of the HPHs. Our results showed that bioHPHs reduce proinflammatory cytokine secretion and gene expression and enhance the expression and release of anti-inflammatory cytokines. In addition, bioHPHs reverse LPS-associated M1 polarization into M2 phenotype.

Conclusions:

These findings open new opportunities for developing nutritional strategies with hemp as a dietary source of biopeptides to prevent the development and progression of inflammatory-related diseases.

P 089 CHARACTERIZATION OF CHANGES IN AMARANTH GRAIN PROTEIN AND PEPTIDE PROFILE AS THE RESULT OF ISOLATION OF PROTEIN AND ITS DIGESTION PROCESS

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Amaranth has aroused special interest due to its agricultural particularities, for its potential application in the functional food industry and for its nutritional characteristics, since the grain was considered by the National Research Council of the USA as one of the most promising foods for this millennium. Amaranth grain contains good amounts of amino acids that are generally limiting in cereals and legumes, such as lysine and methionine, meaning that its composition is close to the profile recommended for human consumption. In addition to its nutritional characteristics, studies have shown antioxidant and hypocholesterolemic activities resulting from the introduction of amaranth protein in the diet. The verification of the occurrence of bioactive peptides in amaranth protein incomplete digestion has been studied in order to promote the incorporation of amaranth grain into a healthy and balanced diet. Considering that the effects of gastrointestinal digestion of peptides are complex and literature about amaranth peptides is scarce, this work was carried out to evaluate the effect of isolation and *in vitro* digestibility over amaranth protein profile, and to characterize amaranth peptides profile by identifying peptide fragments after *in vitro* digestion and after rats' amaranth protein ingestion.

A protein isolate was produced and an *in vitro* enzymatic hydrolysis that simulated the *in vivo* digestion of the amaranth protein was performed. Proximate composition, degree of hydrolysis, electrophoretic and amino acid profiles were verified. The peptide profile of digested amaranth isolate was determined by LC-ESI MS/MS. For rat ingestion assay, male Wistar rats were used. The rats were distributed in groups: basal; amaranth protein isolate administered by gavage; and saline control by gavage. Sequences in amaranth groups that were identical to control groups were disregarded.

Results:

The levels of essential amino acids in amaranth flour and protein isolate are above or similar to that recommended by FAO for maintaining health. The degree of hydrolysis of the protein isolate averaged 65.7%. Little of the electrophoretic profile was modified by the protein isolation processing, but after hydrolysis it was drastically modified. Fractions above 30 kDa were not observed in digested samples. Several peptides were identified after *in vitro* digestion of the amaranth protein isolate, with emphasis on water-insoluble fragments FFSASC and NLLAGYD. In *in vivo* ingestion assay, 16 peptides from plasma and 49 from serum were selected, and some similarities were found among the fragment profile of plasma and serum of rats. The sequence ALGV stands out in rats› blood, presenting low molecular mass and low water solubility and, therefore, has great absorption potential.

We concluded that amaranth protein isolation did not affect amino acid quality of the grain, and *in vitro* digestion was able to break proteins greater than 30 kDa generating many peptides. Major peptides found after amaranth protein *in vitro* digestion differs from peptides found in the blood of the rats, but both experiments presented peptides that deserve a deeper investigation, given their characteristics. Amaranth protein is considered a great source of essential amino acids and may also be applied to produce bioactive peptides.

P 090 PLASMA CIRCULATING AMINO ACIDS IN PRE-DIABETIC PATIENTS AFTER NUTRITIONAL INTERVENTION SUPPLEMENTED WITH HEMP PROTEIN

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Pre-diabetes is the state that precedes diabetes mellitus type II (DMT2). Blood glucose levels are higher than normal subjects, but not high enough to be considered DMT2 and is characterized by a pro-inflammatory state, associated with cardiovascular disease and cancer. The relationship between diet and health has been widely described. The intake of specific groups of food might help reduce inflammation through different pathways.

In this study, a nutritional intervention supplemented with hemp protein in pre-diabetic patients was carried out in order to evaluate whether it has an impact on the circulating amino acids in plasma. Dietary amino acids play significant roles in preventing and treating intestinal inflammation. For 12 weeks, the prediabetic subjects (n=23) ingested hemp protein (10 g/day), and amino acids were measured at the end of the supplementation period.

The results showed that the intake of the hiperproteic diet led to an increase in amino acids such as glutamine, the most abundant one in humans, with several physiological functions, or sarcosine, among others. On the other hand, b-alanine, asparagine, alanine, and 3-metil histidine concentrations were considerably reduced after the 12 weeks. The rest of the nitrogen compounds evaluated did not significantly changed after the 12 weeks of the nutritional intervention.

The amino acids contained in the protein ingested had an impact on the circulating amino acids in plasma of the pre-diabetic patients. It has been reported that glutamine supplementation could improve glycaemic control and levels of incretins, and also that glycine, serine, glutamine, and asparagine are associated with improved insulin sensitivity. These results provide a clear characterization of the amino acids in humans after a hiperproteic nutritional intervention, although diabetic-reduction parameters should be evaluated to consider whether the diet had a positive impact on glucose levels.

P 091 VALORIZATION OF A DAIRY FARM BY-PRODUCT FOR THE ISOLATION OF ANTIOXIDANT PEPTIDES

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Background:

In order to increase the sustainability of the farming system and to reduce waste, it is crucial to find new ways to properly utilize food by-products. Among the potentially interesting components, bioactive peptides have been identified in a wide variety of matrices and are known to exert positive biological effects on our body, such as anti-inflammatory, antihypertensive, antimicrobial and antioxidant activities.

Objectives:

The aim of the research consisted in the isolation of bioactive peptides endowed especially with antioxidant activity from milk permeate, a dairy farm by-product, for their possible future usage in functional food formulations.

Methods:

The peptide-enriched fractions were extracted with chromatography and the peptide sequences identified with mass-spectrometry. Then, the fractions were tested for their antioxidant activity both *in vitro* and in a cellular intestinal model using different approaches.

Results:

The peptide-enriched fractions obtained from milk permeate showed radical scavenging activity *in vitro*. Moreover, in the Caco-2 cell model, the peptide fractions were able to protect from the cytotoxic effect induced by the oxidant compound *tert*-butyl hydroperoxide (TbOOH). In addition, the same fractions antagonized the production of reactive oxygen species (ROS) elicited in cells by TbOOH treatment, further confirming their antioxidant effect.

Additionally, pre-treatment with the peptide fractions was able to rescue the impairment of cellular respiratory capacity induced by hydrogen peroxide in Caco-2 cells when evaluated via Seahorse analysis. Finally, in order to identify the mechanism through which the fractions exert their antioxidant activity, the abundance of several enzymes involved in antioxidant pathways was assessed, resulting in observation of a raise in the expression of specific proteins upon peptide fractions administration to the cell model.

Conclusion:

In summary, peptide fractions endowed with antioxidant activity were successfully isolated from milk permeate. These peptides were shown to act as antioxidants both *in vitro* and in the cellular model. Next, we will identify the specific peptides endowed with the most of the shown antioxidant activity and deepen their mechanism of action in the cell environment. This project is part of the actions finalized at improving the circular economy with potential benefits for both the environment and human health.

P 092 EFFECT OF PROTEIN HYDROLYSATE-ADDED MUFFINS ON GLYCAEMIC INDEX AND FORMATION OF BIOACTIVE PEPTIDES DURING *IN VITRO* DIGESTION

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Background:

Reformulation of food products is a strategy to improve public health. Muffins are a popular bakery product in Western countries that generally have a medium-to-high glycaemic index (GI). Some studies suggest that the use of protein hydrolysates in food may influence GI through several mechanisms. The formation of bioactive peptides (BAPs) from food protein digestion may be implicated in the post-prandial glucose response and several biological activities.

Objectives:

This study aimed to evaluate whether the inclusion of protein hydrolysates in muffins affected starch digestibility and the release of BAPs during the digestion.

Methods:

Eight muffin prototypes were designed and developed by replacing wheat flour with protein hydrolysates from casein, soybean, pea, and rice at two different concentrations so that proteins contributed by 12% (protein source) and 20% (high protein) to the energy value of the product. Each sample underwent a simulated gastrointestinal digestion (INFOGEST method) to explore the potential effect on the postprandial glucose response by assessing the GI and the BAPs (by targeted UHPLC-HRMS) released during the digestion in comparison to a conventional muffin (CM).

Results:

Results showed that muffins added with pea and rice protein hydrolysates had a GI by 15% and 5% lower than CM and during the digestion released peptides with α-amylase-inhibitory activity. On the other hand, casein containing muffins had a GI higher than CM and released opioid BAPs. The profile of BAPs with antihypertensive, antioxidant, opioid, DPPIV- and α-amylase-inhibitor effects released during the digestion of all prototypes will be shown.

Conclusion:

Altogether the results showed that using pea and rice protein hydrolysates as ingredients in muffins leads to products that may reduce post-prandial glucose response affecting starch digestibility; further and multiple effects may be obtained through the release of BAPs during the digestion. *In vivo* studies are needed to validate the effect of the reformulated products on postprandial glucose response and the physiological relevance of BAPs released upon digestion.

P 093 BIOACTIVE PEPTIDE PRODUCTION FROM LENTILS PROTEIN ISOLATE: UNVEILING THE POTENTIAL OF NEGLECTED *Hanseniaspora uvarum* YEAST

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In the current trend where plant-based foods are preferred over animal-based foods, pulses represent an alternative source of protein but also of bioactive peptides (BPs). We investigated the pattern of protein hydrolysis during fermentation of red lentils protein isolate (RLPI) with various lactic acid bacteria and yeast strains. *Hanseniaspora uvarum* SY1 *and Fructilactobacillus sanfranciscensis* E10 were the most proteolytic microorganisms. SY1 led to the highest antiradical, ACE-inhibitory and antifungal activities, as found in low molecular weight water soluble extracts (LMW-WSE). The 2039 peptide sequences identified by LMW-WSE were screened using BIOPEP UWM database, and 36 sequences matched with known BPs. Fermentation generated 12 peptides undetected in raw RLPI. Besides, SY1 led to the highest amounts of BPs, in particular with antioxidant and ACE-inhibitory activities. KVI, LVR, and LVL were detected for the first time in fermented matrices. Forty-four novel potential BPs, worthy of further characterization, were correlated with antifungal activity.

P 094 NOVEL TOOL FOR ENZYMATIC FUCOSYLATION OF OLIGOSACCHARIDES

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Enzymatic fucosylation is carried out by fucosyltransferases *in vivo*. In this reaction, fucosyl is transferred from GDP-Fuc to glycans. Fucose occurs as a component of Lewis antigens, N-glycans, O-glycans, glycolipids, and human milk oligosaccharides. However, *in vivo* fucosylation of glycans requires specific transferases that are selective for their acceptors and the use of expensive nucleotide donors.[1]

To overcome the challenges of *in vitro* synthesis with expensive substrates and donors, transglycosylation catalyzed by glycosidases is an alternative. α-l-Fucosidases are *exo*-glycosidases naturally capable of cleaving α-linked fucosyl residues from glycostructures, where the most common linkages are α(1→2) to galactose and α(1→3/4/6) to *N*-acetylglucosamine residues. They belong to three GH (glycoside hydrolase) families, namely GH29, GH95, and GH151 (http://www.cazy.org/).[2,3] GH95 α-fucosidases are enzymes that act by an inverting mechanism that is highly specific for the cleavage of the α(1→2)-linked fucosyl moiety to galactose at the nonreducing end of oligosaccharides.[4] In contrast, GH29 α-fucosidases use the retaining catalytic mechanism, and their substrate specificity is relatively broad. GH29 α-fucosidases can also synthesize glycoside bonds with cheaper fucosyl donors that contain a good leaving group at C1, such as 4-nitrophenyl fucoside.[5]

This project focuses on the study of fungal α-fucosidases due to the great synthetic capabilities of fungal glycosidases in general. We cultured fungi from the CCF library under different inducers and screened them for extracellular fucosidase activity. We selected two fungi that showed fucosidase activity to characterize the enzyme and determine regioselectivity and synthetic potential.

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P 095 BIOLOGICAL EFFECTS OF PECTIN POLYSACCHARIDES FROM RIPE AND UNRIPE PAPAYA USING 3D COLON CANCER SPHEROIDS AND INTESTINAL ORGANOIDS

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Introduction:

Pectins are a class of highly fermentable dietary polysaccharides found in vegetables and fruits. Several pieces of evidence suggest that pectins may have anti-cancer and anti-inflammatory properties, including the capacity to induce apoptosis, reduce cell viability, regulate cytokine production, and interact with TLRs. However, little is known about the underlying mechanisms by which pectins exert these functions and how the pectin structures influence them.

Objective:

This study was conducted to evaluate the biological effects of pectins in colon cancer 3D spheroids and normal intestinal organoids.

Methods:

Colon cancer spheroids were formed using HT29, HCT116, and NIH3T3 by nanomagnetism. Spheroids were grown for 24 h and then exposed to treatment with ripe and unripe papaya pectins at 0.1 mg/ml for 24 h, 48 h, and 72 h. Cell viability was measured by Alamar Blue and cytotoxicity was by staining with Live/dead solution. After staining, imaging was performed using a fluorescent microscope. The organoid model was generated using Small intestinal stem cells from neonates in a transwell system composed of polarized intestinal epithelial cells. After full differentiation, the inflammation was induced by the addition of *E.coli HS* and the pectin polysaccharides from ripe and unripe papaya at 0.3 mg/ml. After 5 h, cytotoxicity was measured by LDH, IL-6, and IL-8 cytokine concentrations were measured by ELISA in the top supernatant.

Results:

Ripe and unripe papaya pectin treatment for 24 h reduced cancer spheroid viability for HCT116 spheroids only, and ripe pectin treatment for 24 h reduced 3D co-culture spheroid viability for both HT29 and HCT116 models compared to the control *(P<0.05)*. After 48 h, unripe pectins reduced viability for HT29 and ripe for HCT116. Ripe and unripe papaya pectin treatment at 0.1% for 72 h reduced the viability of unicellular HCT116 spheroids *(P<0.05)*. In the co-culture models, HCT116 spheroids had the highest reduction in cell viability. HT29 co-culture spheroids reduced viability only after treatment with ripe papaya pectin *(P<0.05)*. Treatment with unripe pectins for 48 h had the strongest effect on cell toxicity for HCT116/NIH3T3 cancer spheroids. Treatment with only ripe and unripe pectins was not toxic to the intestinal organoids even in the presence of bacteria. Pectin treatment kept the organoids viable and healthy even during exposure to *E.coli HS (P<0.005)*. Both treatments with ripe and unripe papayas pectins alone decreased the production of IL-6 *(P<0.005)*. Adding pectins with the bacteria still reduced the production of IL-6, showing that both pectins modulated the inflammatory response by decreasing the production of the pro-inflammatory cytokine IL-6 *(P<0.005)*. However, no effect was observed for IL-8.

Conclusions:

These results suggest that treatment with native pectin reduces cell viability and induces cell toxicity in colon cancer spheroids and also has potential anti-inflammatory effects, mainly by reducing IL-6, but not IL-8. This could be beneficial in future applications for the treatment of different inflammatory conditions that can lead to cancer and other inflammatory diseases.

P 096 CELL FACTORIES WITH ENGINEERED METABOLIC PATHWAYS FOR PRODUCING HUMAN MILK OLIGOSACCHARIDES

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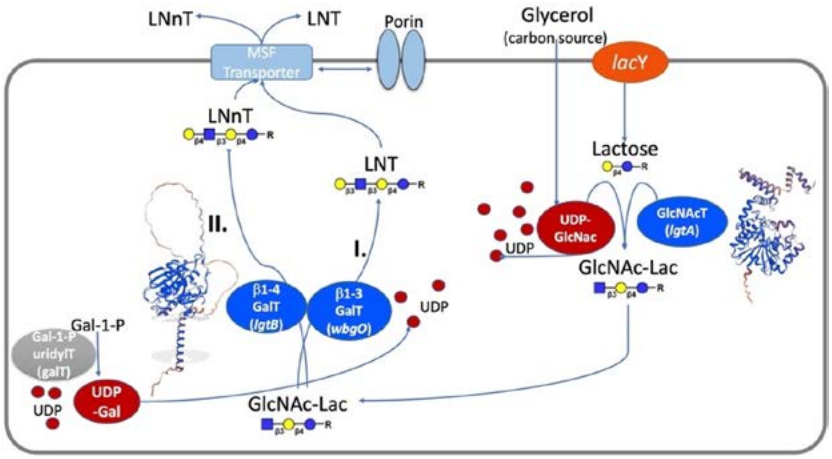
Human milk oligosaccharides (HMOs) are complex compounds responsible for healthy neonatal development of infants with a positive impact on the gastrointestinal microbiome and immune system.[1] Though HMOs are *in vitro* prepared by enzymatic synthesis, this pathway is often complicated by the high price of sugar nucleotides, the low substrate specificity and stability of the enzymes. Production of HMOs *via* genetically-engineered microbial cell factories is a more promising approach.[2] It enables large-scale production in bioreactors with high conversion rates and selectivity. Construction of a functional cell factory comprises establishing new metabolic pathways.[3] The present work shows a large-scale production of lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LN*n*T), the most abundant neutral oligosaccharides in milk, using engineered *Escherichia coli* cell factory. Complementary to the upstream process of production, this work also outlines downstream process of isolation and purification of LNT/LN*n*T.

Fig. 1: Schematic representation of metabolic pathway for production of LNT/LN*n*T.



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P 097 NANOCELLULOSE AND WET-TYPE GRINDER-TREATED OKARA MODULATE GUT MICROBIOTA AND ATTENUATE OBESITY IN HIGH-FAT-FED MICE

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Background:

In recent decades, nanofiber technology has made progress, and a wet grinder (WG) is one of the prominent nanofiber technologies. In our previous study, we atomized okara (soybean residue) using a nanofiber technology and showed that atomized okara effectively suppressed alpha-amylase activity and increased butyrate production by human-dominant intestinal bacteria due to improved dispersibility and viscosity.

Objectives:

We investigated the effect of nanocellulose (NC) and WG-treated okara (WGO) intake on obesity in high-fat diet (HFD)-fed mice. In addition, the gut microbiota composition and plasma metabolites were determined to elucidate the mechanism.

Results:

First, we investigated the effect of NC intake in HFD-fed mice. NC-treated mice received 0.1% or 0.2% NC dispersions in the drinking water for seven weeks. HFD-fed mice weighed more than mice fed a normal fat diet. The weight gain in HFD-fed mice was suppressed in the 0.2% NC-treated group ($p<0.05$) but not in the 0.1% NC-treated group compared to the NC-untreated group. Fat accumulation in epididymal and subcutaneous adipose tissue of HFD-fed mice was also lower in the 0.2% NC-treated group than in the NC-untreated group. Fecal gut microbiota was analyzed by sequencing bacterial 16S ribosomal RNA genes. Oral administration of 0.2% NC, but not 0.1% NC, increased bacterial diversity and induced changes in gut microbiota composition. Principal component analysis (PCA) at the family level revealed a shift in microbiota composition resulting from HFD feeding. This shift in the PC1 axis was reversed by 0.2% NC intake. In addition, the relative abundance of Streptococcaceae and Rikenellaceae decreased while that of Lactobacillaceae increased with higher NC intake. Streptococcaceae and Rikenellaceae have been reported to be associated with metabolic disorders such as obesity and diabetes. Second, we investigated the effect of WGO intake in HFD-fed mice. Consumption of WGO suppressed weight gain and improved glucose tolerance in HFD-fed mice. The WGO-treated HFD-fed mice have higher cecal butyrate levels than other groups of mice ($p<0.05$). Fecal gut microbiota was analyzed by sequencing bacterial 16S ribosomal RNA genes. PCA separated the microbiota composition of the WGO and cellulose-treated HFD-fed mouse groups. With WGO intake, *Ruminococcus* and Lactobacillaceae significantly increased, whereas Rikenellaceae, Bacteroidaceae, and Streptococcaceae significantly decreased in HFD-fed mice ($p<0.05$). In metabolomic analysis, 151 metabolites were increased in WGO-treated HFD-fed mice compared with cellulose-treated HFD-fed mice, indicating that primary bile acid biosynthesis was the pathway significantly upregulated by WGO intake ($p<0.05$).

Conclusions:

Consumption of NC or WGO has inhibitory effects on obesity and diabetes mediated via control of gut bacterial communities. It appears that WGO promotes bile acid excretion as feces and primary bile acid biosynthesis is upregulated.

P 099 MOLECULAR MECHANISM OF STEROL METABOLITES' CYTOTOXICITY IN COLON CELLS: TRANSCRIPTOMIC APPROACH

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Colorectal cancer (CRC) ranks as the third most common and second deadliest cancer. A previous study revealed that cholesterol metabolites produced by gut microbiota induce apoptosis and G_0/G_1 cell cycle phase arrest in non-tumor colon cells. On the contrary, the metabolite derived from β-sitosterol (the main dietary plant sterol) is apoptotic in colon tumor cells and protect non-tumor cells from the deleterious effect of the most cytotoxic cholesterol metabolite (cholestenone) [1]. Since the cytotoxic pathway is unknown, the present study aims to investigate the mechanism of action of cholesterol and β-sitosterol metabolites using a transcriptomic approach. The changes in the expression of apoptosis and cell cycle-related genes were measured in non-tumor (CCD-18Co) and tumor human colon cells (Caco-2) after exposure (6 h, 75 μM) to metabolites derived from cholesterol (coprostanone and cholestenone) and β-sitosterol (ethylcoprostanol) using quantitative PCR. To assess the impact of ethylcoprostanol on colonic cell damage induced by cholestenone, co-treatment of both compounds was tested. Cholesterol metabolites increased (*vs*. control) the expression of genes related with intrinsic (*BAX/BCL2* ratio and *CASP9*) and extrinsic pathway (*CASP8*) of apoptosis. These changes, in general, occurred predominately in non-tumor cells compared to tumor cells (1.6–2.1 *vs*. 1.4–1.7-fold). The selective cytotoxicity of cholesterol metabolites in non-tumor cells was confirmed by the increased expression of the *CASP3* gene only in this cell line (1.3–1.4-fold). On the other hand, an arrest of the cell cycle in non-tumor cells occurred at the initial stage of the G_1/S transition, since a reduction in the expression of the gene encoding cyclin E_1 was observed (0.5–0.7-fold). Furthermore, no changes in the expression of the p21 and p53 genes were noted, ruling out their mediation in the effects of cholesterol metabolites. Regarding ethylcoprostanol, its cytotoxicity seems to occur due to intrinsic apoptosis, as deduced from the increase in the *BAX/BCL2* ratio observed only in tumor cells (1.5-fold). This apoptotic effect was accompanied by a reduction in the gene expression of the antiapoptotic protein p21 (0.7-fold), although independently of the p53 gene. Selective cytotoxicity on tumor cells was confirmed by a reduction in the expression of the cyclins D_1 (0.4-fold) and E_1 (0.7-fold) encoding genes, fact that did not occur in non-tumor cells. In addition, ethylcoprostanol showed a protective effect on cholestenone-induced gene expression changes on non-tumor cells. In this sense, the increase in the gene expression of the *BAX/BCL2* ratio was attenuated (1.6 *vs*. 1.2-fold) and the expression of the *CASP3* and *CASP9* genes was restored to the control value (*vs*. treatment with cholestenone). In conclusion, cholesterol metabolites could act as promoters of CRC through their cytotoxic activity on non-tumor colon cells, while ethylcoprostanol is a promising candidate as a therapeutic adjuvant in CRC due to its selective cytotoxicity on tumor cells and cytoprotection in non-tumor cells.

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P 100 VARIABILITY OF LIPIDS IN HUMAN MILK AND COW MILK BASED FORMULA

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Human milk lipids are the best source of energy for infants during early development, particularly in the first six months of life. Glycerides are present in the core of milk fat globules, additionally, glycerophosphatides, sphingolipids and glycolipids are located in the milk fat globule membrane. Glycerides (mainly triacylglycerols (TAGs)) account for approx. 98–99% of the total amount of milk lipids and account for up to 50% of the energy necessary for breastfed infants. Even though human milk is considered as golden standard, in some cases breastfeeding is impossible. Therefore, a thorough understanding of the composition of human milk, as well as the concentration, structure and function of individual components, including lipids, is very important. With the progress in this knowledge, it will be possible to develop infant formulas that better mimic human milk for infants for whom it is the only possible option of feeding or for those who are supplemented.

TAGs are the major components of human milk and milk formulas and their nutritional and functional properties are affected by the composition and positional distribution of fatty acids (FA). Palmitic acid (PA), which is found in the sn-2 position of TAGs in most mammalian milks, is the dominant acid in this position in both human and other mammalian milks, but its amount in animal milks is significantly reduced due to competition for myristic and oleic acids. Additionally, the concentration of TAGs with unsaturated fatty acids and even-chain functional fatty acids, such as arachidonic acid (AA) and docosahexaenoic acid (DHA), is higher in human milk than that in other mammalian milk. Also with regard to polar lipids the concentration of PL-PUFAs in mammalian milk is too low and it is impossible to simulate human milk PLs with any single kind of mammalian milk.

Ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) is currently the key analytical method used to characterize lipids. Human milk lipids are composed of lipid subclasses defined by the structures (carbon number, number and position of double bonds) and positions of fatty acyls. To date, most of the research has focused on the main ingredients of human milk and other mammalian, i.e. TAGs. It has been shown that, apart from the variability in the circadian cycle, human milk is characterized by individual variability. Moreover, the use of different methods, apparatus and the method of sample preparation makes it difficult to compare findings of different authors.

The aim of the study was to develop a methodology for testing samples of human milk and infant formulas using Shimadzu Nexera-2 liquid chromatograph coupled with the SCIEX TripleTOF® 6600+ mass spectrometer. Conducting preliminary research will allow for an in-depth study of the composition and variability of the milk of Polish lactating women and infant formulas available on the Polish market. The results can be useful in designing new milk formulas that better mimic human milk.

P 101 CONSUMING MARGARINES AND/OR YOGURTS PROVIDING RECOMMENDED OR HIGH DOSES OF PLANT STANOL ESTERS DOES NOT AFFECT *EX VIVO* T-CELL DERIVED CYTOKINE PRODUCTION IN HEALTHY VOLUNTEERS

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Background:

The immune system is a highly controlled network that can efficiently maintain a state of immunological balance known as immune homeostasis. However, a disbalance may occur, leading to under- or overactivity of the immune system. In fact, overactivity of T-helper (Th)2 cells has been linked to asthma. Previous *in vitro* studies have now shown that plant stanol esters can modify the T-helper cell response in individuals with an imbalance in Th1/Th2. For example, plant stanol ester consumption increased the activity of regulatory T-cells and the production of the Th1 cytokines IFNγ and IL-2 in asthma patients. However, whether plant stanol consumption affects cytokine production in healthy subjects without a skewed Th1/Th2 cytokine balance is unknown.

Objective:

To examine the effects of recommended (2.5 gr/day) or high-dose (9.0 gr/day) plant stanol intake on the Th1/Th2 cytokine balance in healthy subjects.

Methods:

In two randomized controlled trials, peripheral blood mononuclear cells (PBMCs) were isolated from apparently healthy participants, cultured and stimulated with 5 µg/ml Phytohemagglutinin-M to evaluate effects on *ex vivo* cytokine production. In both studies, PBMCs were isolated at the end of the run-in and intervention periods. In the first study, twenty participants (n=10/n=10) consumed plant stanol ester enriched margarines (2.5 gr/day) or control margarines for three weeks after a one-week run-in period. IFNγ and IL-4 in the culture medium were quantified using sandwich ELISAs. In the second study, nineteen participants (n=9/n=10) consumed plant stanol ester enriched margarines and yogurts (9 gr/day) or control products for four weeks after a three-week run-in period. Cytokine (IL-2, IL-12, IFNγ, IL-4, IL-13, IL-10, and IL-17) concentrations were measured in culture medium using a custom electrofluorescence cytokine multiplex assay. A Th1/Th2 index was calculated using standardized values of IFNγ and IL-2 versus IL-4 and IL-13. Serum lipids and non-cholesterol sterols were measured in both studies.

Results:

In both studies, compliance was confirmed by significant increases in serum total cholesterol (TC)-standardized sitostanol and campestanol levels. None of the changes in *ex vivo* cytokine production differed between groups. The Th1/Th2 index also remained unaffected. In the first study, TC tended to decrease (-0.48 [-1.00; 0.02] mmol/L; $p=0.06$) compared to control. In the second study, TC (-0.82 [-1.19; -0.44] mmol/L; $p<0.001$) and LDL-C (-0.77 [-1.11;-0.42] mmol/L; $p<0.001$] significantly decreased and triglycerides tended to decrease compared to control (-0.18 [-0.37; 0.01]; $p=0.07$).

Conclusion:

The effects of daily plant stanol ester consumption in healthy subjects (both at a recommended intake of 2.5 gr/day as well as an extremely high intake of 9.0 gr/day) did not alter the Th1/Th2 balance as measured by *ex vivo* cytokine production. This indicates that consuming plant stanols by a healthy population does not induce an immune skewing response. We speculate that plant stanols only exert effects when there is already a Th1/Th2 imbalance (e.g., asthma). Therefore, the underlying mechanisms of plant stanol-induced effects on the immune system under skewed conditions should be further investigated.

P 102 CHEMOPREVENTIVE EFFECT OF COLONIC FERMENTATION SUPERNATANTS OF A WHOLEMEAL RYE BREAD WITH/WITHOUT PLANT STEROLS IN COLON ADENOCARCINOMA CELLS

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Colon cancer is one of the most prevalent diseases in developed countries. Plant sterols (PS) have shown a chemopreventive effect at colonic level due to its low absorption. This effect has been previously tested in colon tumor cells (Caco-2) and non-tumor cells (CCD-18co), exposed to standard solutions of PS and their metabolites [1,2].

The objective of this work is to evaluate the chemopreventive effect of a PS-enriched wholemeal rye bread (1.8 g PS/portion) compared to a non-enriched one, after *in vitro* digestion and fermentation.

Breads were digested and fermented with a dynamic multi-compartmental continuous digestor (simgi®, CIAL-CSIC), obtaining digestion blanks and fermentation liquids (FL). Samples were centrifuged, diluted with DMEM, and filtered, obtaining fermentation supernatants from stabilization blank (SB), FL without PS (FL0), wash blank (WB), and FL with PS (FLPS). All flow cytometry assays with colon cells were performed after 24 h of treatment (apoptosis, cell cycle progression, ceramide, reactive oxygen species (ROS), reduced glutathione (GSH), and calcium).

After previous cell viability screening with the MTT assay, dilution 1/5 (v/v) was selected, and only Caco-2 cells were used since the FL did not affect the viability of CCD-18co cells. A reduction in % of viable cells was observed in both FL compared to their blanks (21 and 35 % in FL0 and FLPS, respectively). Besides, early apoptosis was increased (by 20 % in both FL), but late apoptosis was significantly ($p<0.05$) more increased with FLPS *vs.* FL0 (47 *vs.* 9 %). Furthermore, an increase in SubG1 population was observed in both FL (2.3- and 2.2-fold increase in FL0 and FLPS, respectively), with a subsequent reduction of G0/G1, S, and G2/M phases, supporting the apoptotic effect of the FL. Ceramide (pro-apoptotic) levels were lower in FL *vs.* blanks, implying some cytotoxicity from compounds present in blanks. ROS levels were also lower in FL, but the reduction in FLPS was scarcer (35 *vs.* 65 % of blank in FL0 and FLPS, respectively), suggesting a higher apoptotic effect ROS-mediated in FLPS. However, GSH levels differed from these results, showing a higher reduction in FL0 (64 % of blank) compared to FLPS (82% of blank). This trend was also observed in calcium levels, where an increase was observed in both FL, higher in FL0 (234% of blank) than in FLPS (127% of blank).

These results show a chemopreventive effect of rye bread FL, due to their apoptosis induction in tumoral colonic cells, without affecting non-tumoral cells. However, although it seems that the PS exert an extra positive effect, all parameters did not show the same trend and it would be necessary to confirm it with subsequent gene expression or proteomic assays and additional data of antioxidants and short chain fatty acids.

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Acknowledgements:

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P 103 EXPLORING THE RELATIONSHIP BETWEEN PLANT STEROL BIOACCESSIBILITY AND LIPOLYSIS OF ENRICHED BREAD IN SENIOR DIGESTION CONDITIONS

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Plant sterol (PS)-enriched foods are considered a valuable tool for the prevention of cardiovascular diseases, especially in senior population (>65 years). Despite its relevant effect on human health, there are few studies on the fate of food and PS during digestion under senior adult conditions[1,2]. The aim of this study was to determine the PS bioaccessibility (BA) and lipolysis from a PS-enriched wholemeal rye bread under senior adult conditions. The bread was subjected to *in vitro* digestion under older adult conditions (reduction of enzyme activity and agitation, modification of pH and increase of digestion time) at (i) gastric step, (ii) gastric and intestinal step, and (iii) INFOGEST 2.0 method with cholesterol esterase as control. The PS content in bread and in the obtained bioaccessible fractions (BF) were determined by gas chromatography-mass spectrometry (GC-MS), in order to calculate the BAs[3]. The main lipid classes in both the bread and BF were determined by GC-flame ionization detection (FID)[4]. The total PS content in the bread was 1.6 g/100 g, with b-sitosterol (81.3%) > campesterol and sitostanol (6.8%) > campestanol (1.7%) > stigmasterol, Δ^5-avenasterol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol and Δ^7-avenasterol (<1%). The BA of total PS after the control digestion was 11.6%, with sitostanol as the most bioaccessible sterol (18.5%). The modified gastric step conditions led to a higher BA of total PS (values of 16.1%), with sitostanol remaining as the most bioaccessible sterol (23.1%). The total PS BA obtained with the gastric and intestinal adaptations was not significantly different with respect to that of the control (9.9% of BA), with sitostanol and stigmasterol displaying the highest BA (16.6 and 15.6%, respectively). The lipid profile in the bread confirmed that free sterols were the major lipid class (82.1%, in agreement with the PS enrichment of the bread), followed by triacylglycerols (12.3%), and free fatty acids, diacylglycerols, monoacylglycerols, esterified sterols and tocopherols (<1.6%). The total lipid profile obtained in all digestion conditions here tested showed a predominant abundance of free fatty acids (43.2–48.2%) and free sterols (47.2–51.2%). In this case, significant differences were observed only in the intestinal phase, where a higher abundance of esterified sterols (8.8%), diacylglycerols (31.7%), and triacylglycerols (67.1%) was found in the modified condition with respect to the control method. Therefore, our study suggests that senior digestion conditions do not significantly affect the BA of PS in PS-enriched bread, despite the decrease in lipolysis. However, further studies are needed to confirm these results for a better understanding of PS fate in aging populations.

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P 104 *IN VITRO* DIGESTION BEHAVIOR OF LIPIDIC COMPLEXES

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In the last decade, the food industry has faced a significant revolution towards healthier, sustainable and natural products. In this scenario, functional and natural products based on bioactive compounds stand out. Fatty acids (FAs), such as Omega FAs, have been widely applied in food industry as bioactive compounds and/or functional additives in their free, salt, ester and other forms. The development of new forms of FAs by combining them with other bioactive compounds (e.g. ionic liquids) could be a promising alternative strategy to improve their physical and bioactive properties and delivery. The aim of this work was to study the *in vitro* digestion behavior of lipidic complexes derived from FAs. Complexes of lipid compound (oleic acid, OLE or linoleic acid, LIN) and lysine were prepared and submitted to *in vitro* digestion using static protocol. Samples were collected after gastric and intestinal digestion. Free FA (FFA) content was determined by using a Varian 3800 gas chromatographer equipped with a flame ionization detector (FID) and a Teknokroma TRB-WAX column (Teknokroma Analítica, Barcelona, Spain). FFAs were esterified in the presence of HCl/1-propanol (25:75, v/v) at 100 °C for 3.5 h and propyl-esters were extracted with dichloromethane. After gastric phase digestion, approximately 67.30% and 56.29% of FFAs were released for formulations prepared with OLE and LIN, respectively. However, after intestinal phase digestion, the realeasing of FFAs reached values close to 78.61% and 80.63% for formulations prepared with OLE and LIN, respectively. Depending on the digestion phase, samples presented different FFA content possibly related to the presence of acid fluid in case of gastric and alkaline fluid in case of intestinal phase. These results open perspective of application of FA-based systems for the development of functional products for the food industry.

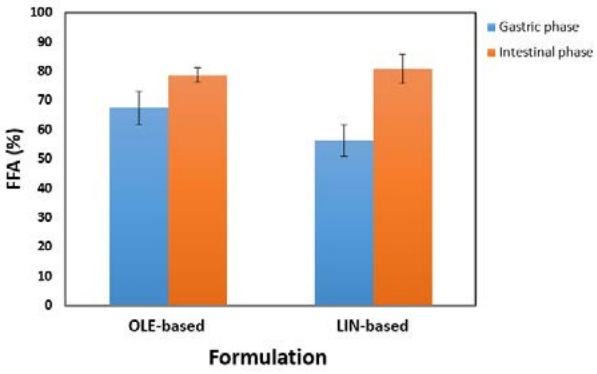

Figure 1: Free fatty acid (FFA) content for oleic acid (OLE) or linoleic acid (LIN) based formulations.

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P 105 ASSESSMENT OF DHA INTAKE BY BREASTFEEDING WOMEN

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Diet and lifestyle are important for both mother and child, already in the preconception period, throughout pregnancy and breastfeeding. One of the most valuable ingredients for the proper development of infants is docosahexaenoic acid (DHA). DHA is a long-chain (LC) omega-3 polyunsaturated fatty acid that is essential for fetal brain and retinal development during pregnancy. In addition, it is necessary for the proper psychomotor neurodevelopment in the first months of an infant's life, where it is supplied in large amounts with maternal milk. DHA can be synthesized from the precursor, α-linoleic acid (ALA), however, due to the ineffective conversion of ALA to DHA, DHA supplementation and the consumption of products rich in it by pregnant and breastfeeding women are recommended. One of the main sources of DHA in the daily diet is fish, the consumption of which is generally not high, especially in non-Mediterranean countries. Therefore, it is important to assess DHA intake by pregnant and lactating women. The study using an online questionnaire was conducted among Polish breastfeeding women. In this study, a food frequency questionnaire was used to assess DHA intake by breastfeeding women [1,2]. The questions mainly concerned the consumption of DHA-rich products, i.e. fish, seafood, poultry, egg yolks and liver. The survey also included questions about DHA supplementation. The daily DHA intake of each study participant was calculated using a questionnaire. The conducted survey indicates the diversification of DHA intake among breastfeeding women, especially due to low or high consumption of fish and supplementation or lack of it. Moreover, not all study participants supplemented DHA during pregnancy, and not all of them were informed by their physician or obstetrician about the possibility of supplementation. Therefore, it is necessary to increase the nutritional education of pregnant and breastfeeding women and greater support from dieticians, doctors and obstetricians for the consumption of DHA, which plays an important role in the diet of breastfeeding women.

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P 106 OCCURRENCE AND DIETARY EXPOSURE ASSESSMENT OF HEAVY METALS IN BABY FOODS IN THE KINGDOM OF SAUDI ARABIA

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Early childhood exposure to heavy metals arsenic (As), cadmium (Cd), and lead (Pb) through baby foods unfolds many concerns about their toxic effects on growth and health. In this study, occurrence and dietary intake of As, Cd, and Pb in stage 1 infant formulae (0–6 months), stage 2 infant formulae (7–12 months), cereal-based meals, and biscuits were estimated. Firstly, the levels of As, Cd, and Pb were determined with ICP-MS, followed by the calculation of estimated daily intake (EDI), target hazard quotient (THQ) and hazard index (HI) for As and Cd, and margin of exposure (MoE) for Pb. Mean levels of As, Cd and Pb were the highest in cereal-based meals and biscuits as 15.5–11.1, 5.18–8.76, and 35.2–53.8 µg/kg, respectively. Newborns to 6 months old infants were estimated to be the highest exposed population to Cd and Pb (0.08 and 0.36 µg/kg bw/day), while infants aged 7–12 months old were exposed the highest to As. Based on the THQ, HI and MoE findings, the current exposure levels from the selected baby foods to As, Cd and Pb pose low potential chronic risks to both infant age groups. This research provides a roadmap for future investigations in chemical contaminants often detected in baby foods consumed regularly by Saudi infants.

P 107 OCCURRENCE AND RISK ASSESSMENT OF 3-MONOCHLOROPROPANEDIOL (3-MCPD) AND GLYCIDYL FATTY ACID ESTERS (GE) IN INFANT FORMULA AND BABY FOOD PRODUCTS ON THE SAUDI MARKET

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Introduction:

3-Monochloropropanediol fatty acid esters (3-MCPDE) and glycidyl esters (GE) are newly identified processing-induced chemical toxicants. Their presence in infant formula and baby foods has been reported widely in the literature. This study aimed to analyze the levels of 3-MCPDE and GE in infant formulas and baby foods available in Saudi Arabia, followed by estimated dietary risk assessment for infants and young children from birth to 3 years.

Methodology:

Therefore, a total of eighty-five different commercial infant formulas (n=35) and baby foods (n=50) available for consumption by infants and babies aged 0–3 years old, were purchased from the Saudi market during 2022. The samples were comprised of powder infant formulas (0–6 months), follow-up formulas (6–36 months), cereal-based products, vegetable and fruit puree baby foods, puffs and biscuits. The samples were analyzed for the first time for the occurrence of 3-MCPDE and GE using a gas chromatography-tandem mass spectrometry method (GC-MS/MS).

Results and Discussion:

The results revealed that 3-MCPDE were observed in all analyzed samples with mean concentrations ranging from 29.46 to 115.1 µg/kg. Among the different brands, the highest concentration was found in 0–6 months formulas at 285 µg/kg, while the lowest concentration was observed in fruit purees at 2 µg/kg. The percentage of non-compliant samples compared to the maximum limits set by the European Union was 9.4% (n=8), six of them were 0–6 month formulas while the two other samples were 6–12 formulas. GE was detected in almost 80% of the samples with mean concentration ranging from 0.004 to 0.045 mg/kg. The highest concentration was also found in 0–6 months formulas at 0.217 mg/kg. The study is still in the process of analyzing the data and conducting the dietary risk assessment, which will be presented in full in September. The data indicate an increase in dietary exposure to these contaminants in the 0–6 months age group fed infant formula exclusively. However, the exposure declines as more food sources are introduced to the babies.

Conclusion:

The findings suggest that priority should be given to controlling the contamination level of edible oil added to infant formulas and baby foods. Since the obtained results present the first national report of 3-MCPDE and GE levels, this study could be of great interest for establishing a new standard covering maximum levels for these contaminants in several food items. We suggest setting maximum levels for these contaminants in edible oils used for infant foods manufacturing.

P 108 OCCURRENCE AND RISK ASSESSMENT OF 3-MONOCHLOROPROPANEDIOL AND GLYCIDYL FATTY ACID ESTERS IN INFANT FORMULA AND BABY FOOD PRODUCTS ON THE SAUDI MARKET

Lama Almaiman[1], *Jumanah Alamir*[1], *Faisal Albuhayjan*[1], *Rawdah Akamsiei*[1], *Nujood Alessa*[1], *Mohammed Alhuthiel*[1], *Mohammed Beneid*[1]

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P 109 CHARACTERISTICS OF *Clostridium perfringens* ISOLATES FROM VARIOUS MEATS IN SOUTH KOREA

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Background:

Clostridium perfringens is a Gram-positive spore-forming anaerobe that is the causative agent of many histotoxic and enterotoxic diseases in humans and animals. The key feature of these diseases is that they are mediated by the production of potent toxins, most of which are extracellular. These toxins generally are involved in specific disease syndromes. For example, α-toxin is essential for human clostridial myonecrosis or gas gangrene, *C. perfringens* enterotoxin is required for human food poisoning, β-toxin is essential for specific enteric *C. perfringens* infections in several species, ε-toxin is the key toxin in many enterotoxemic *C. perfringens* infections in sheep and goats and NetB toxin is essential for necrotic enteritis in chickens.

Objectives:

The aims of this study is to investigate the presence of *C. perfringens* from various meats, to assess the presence of toxin genes in the isolates, and to investigate the antibiotic susceptibility of the isolates.

Methods:

One hundred fifty three, forty seven, nine and three *C. perfringens* were isolated from pigs with necrotic enteritis, beef, pork, and chicken meats, respectively. Presence of toxin genes including *cpa, cpb, cpe, etx, iap* and *netB* genes in the isolates were tested using PCR. Antimicrobial resistance for all *C. perfringens* isolates was tested using the disk-agar method standardized by the Clinical and Laboratory Standards Institute. The isolates were tested against a panel of 19 antimicrobials: Amikacin (AK), Gentamicin (CN), Kanamycin (K), Ampicillin (AM), Amoxacillin-clavulanic acid (AmC), Penicillin (P), Ceftriaxone (CRO), Cephalothin (CF), Vancomycin (VA), Tetracycline (Te), Erythromycin (E), Chloramphenicol (C), Enrofloxacin (ENR), Ciprofloxacin (CIP), Lincomycin (MY), Bacitracin (B), Cefotaxime (CTX), Metronidazole (MTZ), and Nalidixic acid (NA). Results were obtained after incubating samples for 24 h at 37°C under strict anaerobic conditions and were interpreted according to CLSI.

Results:

All the 212 isolates were toxin type A, and were susceptible to 4 of 19 antimicrobials tested in this study, Ceftriaxone, Vancomycin, Metronidazole and Chloramphenicol. However, all 212 isolates were resistant to at least 1 of other 14 antimicrobials. Furthermore, 153 (70.8%) isolates were resistant to 2 or more antimicrobials. A total of 103 among 153 isolates from pigs were resistant to at least 1 antimicrobial. All isolates from meats were resistant to at least 1 antimicrobial. Three isolates (2.9%) of the 103 isolates resistant to antimicrobials were resistant to 11 antimicrobials, three (6.4%) and one (11.1%) isolates from beef and pork meats were resistant to 9 antimicrobials, and one isolate (33.3%) from chicken meat was resistant to 8 antimicrobials. Profiles of antimicrobial resistance revealed 24 resistance types. The isolates were primarily resistant to amikacin, kanamycin, enrofloxacin, nalidixic acid and tetracycline. Furthermore, all the 103 isolates from the pigs were resistant to enrofloxacin, however, all isolates from beef, pork and chicken meats were resistant to penicillin.

Conclusion:

These results indicate that *C. perfringens* toxin type A was prevalent in industrial animals of Korea. The high rate of resistance in *C. perfringens* isolates, sometimes to multiple drugs, may complicate future options for treating human infections.

P 110 CASCADE ENZYMATIC REACTIONS FOR ASSAY OF FREE CYANIDE

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Free cyanide (fCN), a well-known highly toxic substance, is present in the environment due to industrial activities and plant metabolism. Dozens of plants, among them a number of food plant species, contain cyanogenic glucosides as a defence strategy against herbivors. Free cyanide is produced during the decomposition of cyanogenic glycosides, specifically their aglycons, nitriles. Consumption of food prepared from cyanide-containing plants such as the tropical agricultural crop cassava (manioc, tapioca) may be risky, unless the cyanide content is decreased to tolerable levels by plant work-up. In addition, many food plants widely cultivated in temperate-zone regions contain non-negligible levels of free cyanide, which may be also present in the final products (orange or sour cherry juices, almond products etc). Consumption of plant parts that contain excess free cyanide such as apricot kernels must be avoided.

Cyanide poisoning can occur by exposition to cyanide through dermal tissue, lungs, intestinal tract and even through eyes. In the body, cyanide causes chemical asphyxiation by iron binding and respiratory chain inhibition. Accute cyanide poisining results in damaging lungs, heart, nervous and endocrine systems. Human body can, to some extent, metabolise cyanide on its own but a long-term exposure leads to demyelination of periferal nerves, thyroid gland damage, optical neuropathy and deafness, although cyanide does not accumulate in human body. The latter chronical effects occur e.g. as a result of consuming unproperly treated cassava.

Thus, the wide occurrence of natural but also man-made cyanide requires to monitor and control the levels of this substance in various materials. Reliable and fast methods to detect cyanide are needed for food control as well as for the analysis of wastewater, surface water or soil samples. Unlike physico-chemical methods, which are commonly used for cyanide detection, enzymatic methods can be more rapid, selective and sensitive. Some methods used halohydrin dehalogenase catalyzing reaction with butylene oxide, with products quantitatively detected by gas chromatography. Enzymatic cyanide detection can be also performed via biosensors based on amperometry (tyrosinases) or conductometry (horseradisch peroxidase).

Many spectrophometric assays of small molecules are based on the use of enzymes that produce or consume NADH but such methods have been largely missing for fCN. Here, we proposed spectrophotomeric fCN assays based on enzymatic cascades. fCN was hydrolyzed to formic acid by CynDs or by a cascade of cyanide hydratases (CynHs; EC 4.2.1.66) and formamidase (AmiF; EC 3.5.1.49). In both cases, the formic acid formed was dehydrated by FDH with concomitant NAD reduction (Scheme 1). NADH was determined at 340 nm. Alternatively, formamide consumption and formic acid production were determined by HPLC, but with a lower sensitivity. The methods were optimized in terms of fCN range (sample dilution), pH (9.0–10.0) and reaction time, using model samples. Testing method suitability for real samples such as plant material extracts is in progress.

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P 111 EVALUATION OF THE SAFETY OF SIX MYCOTOXINS ACCORDING TO FOUR *IN VITRO* ASSAYS USED FOR THE CHARACTERIZATION OF GENOTOXICITY / MUTGENICITY

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Mycotoxins are a common contaminant of agricultural crops that have acute toxic effects on the human organism. Moreover, long-term consumption of even smallamounts allows for manifestations of its genotoxicity with serious consequences. Authorities focus mainly on the acutely toxic mycotoxins, but others that are not that toxic, such as members of the ergot alkaloid group, are not even characterized properly from a genotoxic point of view. Currently used *in vitro* methods for genotoxicity testing are time consuming and not suitable for high-throughput testing. More methods are needed to detect various mechanisms of activity. We present an overview of six known mycotoxins, mainly ergot alkaloids (beauvericin, zearalenone, ergocornin, ergocristine, α-ergocriptine and ergosine), for which their genotoxic effects are not known or are ambiguous. We compare the results of three tests recommended by the authorities (Ames test, HPRT, and comet assay) and one high-throughput immunofluorescence method using histone H2AX phosphorylation, which could make genotoxicity measurements more efficient. At the same time, we used extracellular metabolic activation (mouse liver homogenate) in all tests to detect potential promutagens. All assays using tissue cell lines indicated the genotoxicity of beauvericin. For other mycotoxins, the ambiguous results obtained were probably due to a different mechanism of action. In conclusion, for the correct detection of genotoxic substances, it is necessary to combine the methods appropriately and prioritize the *in vitro* testing on tissue cells, which can reduce false negative/positive results.

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P 112 REDUCTION OF THE EXPOSURE TO THE CONTAMINANTS FURAN AND ALKYLFURANS BY BREAKFAST CEREALS

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Furan and the alkylfurans 2-methylfuran, 3-methylfuran, 2,5-dimethylfuran, 2-ethylfuran and 2-pentylfuran are possibly carcinogenic, volatile compounds known as process contaminants in food [1]. They are formed during thermal processing steps such as roasting, baking, extruding or toasting. Furan and alkylfurans can be generated from a wide range of precursors like carbohydrates, amino acids, unsaturated fatty acids, ascorbic acid and carotenoids. Although the highest levels of these compounds (especially 2methylfuran) have been determined in coffee, the exposure through breakfast cereals is of particular importance as these are often consumed in large quantities and, moreover, to a large extent by children [2].

Using a validated headspace gas chromatography-mass spectrometry method with solid phase micro extraction (SPME) for the detection of furan and alkylfurans in breakfast cereals, a comprehensive data collection on the occurrence of furan and alkylfurans in breakfast cereals was carried out. Levels of up to 230 µg/kg of furan and up to 600 µg/kg of total furan (including alkylfurans) were determined, particularly for the thermal processing steps of puffing of cereals, extrusion cooking and roasting during the production of flakes and cornflakes. Furan, 2methylfuran and 2-pentylfuran could be identified as the most abundant analytes in the samples. Subsequently, breakfast cereal samples were evaluated for their production type as well as ingredients like grains, sugars or fat. Additionally, acrylamide levels were determined for a potential correlation analysis.

To study the furan formation in detail, samples from different production steps of breakfast cereal manufacturing were analyzed to monitor the furan and acrylamide formation in these steps. The obtained results were compared with data from model experiments to further characterize critical steps of furan and alkylfuran formation in breakfast cereals. Therefore, different kinds of breakfast cereals were prepared in model experiments under systematically varied processing conditions. Based on these results, raw materials, specific mechanical energy uptake, processing temperature and moisture level could be identified as critical parameters for the formation and thus for the exposure to furan and specific alkylfurans via breakfast cereals.

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P 113 *IN VITRO* SUPPORTIVE EFFECT OF OLIVE BIOACTIVES ON MYCOTOXIN CHALLENGED INTESTINAL CELLS (IPEC-J2)

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Deoxynivalenol (DON) is one of the most frequent mycotoxins found in cereals, grains, and seeds.[1] Low levels of DON are reported to induce adverse effects on the intestinal epithelium of farming animals and are considered one of the main threats currently present in animal production. Even though some additives can be used to decrease the negative effects of mycotoxins present in feed, mainly toxin binders, their effectivity is limited. Olive bioactives (polyphenols and triterpenes) have displayed potent anti-inflammatory and anti-oxidant activities, supporting animals' homeostasis under immunological challenges in both, swine, and poultry.[2,3] However, no evidence has been published about their potential benefits against a mycotoxin challenge.

The aim of this study was to evaluate the protective effect of a standardized mixture of olive bioactives (OBE) on an IPEC-J2 cell monolayer disrupted by DON. IPEC-J2 cells were cultivated on 24-well cell culture inserts until day 6 at 37 ºC and 5% CO_2 with DMEM:F12 medium supplemented with 5% porcine serum (PS) and antibiotics (P/S). On day 5 cells were treated either with media (CON group) or with DON (40 µM; DON group) to analyze the effect of the mycotoxin over the barrier. The benefits of olive bioactives were assessed by pre-incubation of the monolayers with a standardized mixture of olive extracts containing >0.3% of polyphenols and >2.5% of triterpenes (50 µg/mL; Lucta S.A.) for 24 h before DON application (DON+OBE group). Transepithelial barrier resistance (TEER) was used to evaluate the barrier status and four groups of gene markers associated with inflammation (IL-8, IL-6, IL-10 and TNF-alpha), epithelial barrier (occluding, ZO-1, Claudin-3, Claudin-4 and E-Cadherin), oxidative stress (COX-2, GPx1, GPx4 and MnSOD) and cell transport (Glt2 and SLGT1) were measured by qPCR to elucidate the mode of action.

The negative effect of DON over the monolayer was evidenced by a reduction in 50% of the TEER, associated with the induced over-expression of genes associated with inflammation (IL-6, IL-8 and IL-10), oxidative stress (GPx1 and MnSOD) and nutrient transport (GluT2), in DON group compared to CON ($P<0.05$). OBE (OBE+DON) pre-treatment was able to significantly prevent the TEER reduction compared to DON group ($P<0.05$). This effect was accompanied by the downregulation of IL-8, IL-6 and IL-10 inflammatory cytokines ($P<0.05$) and a tendency to reduce the GluT2 overexpression in the DON+OBE group compared to DON ($P<0.05$).

In conclusion, olive bioactives can support the integrity of the intestinal epithelial barrier against the disruptive effect induced by DON in an IPEC-J2 model and may be a potential new strategy to improve animal resilience to the negative effects caused by the presence of mycotoxins in feed. The anti-inflammatory effect exerted by the olive extracts may be mechanistically involved in the displayed benefits.

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P 114 MATERNAL DIET QUALITY AND HEALTH STATUS OF NEWBORNS

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Persistent organic substances (POPs) are compounds of mainly anthropogenic origin that persist in the environment for a long time, become part of food chains, and some of them accumulate in living organisms. Due to the adverse effects on organisms, these substances are being monitored and the use of some of them has already been banned or at least significantly restricted (Lallas, 2001).

In our study, we analyzed the diet of 53 pregnant women from two regions of the Czech Republic with different levels of air pollution. The women recorded in detail all the food they ate for one week during the last month of pregnancy, and at the same time collected a quarter of this food in boxes (one box for one day). From the dietary records, we obtained information about the quantity and quality of the diet and determined the concentrations of 67 different persistent organic pollutants in the collected samples. These pollutants belong to five different groups – polychlorinated biphenyls, organochlorine pesticides, brominated flame retardants, perfluorinated compounds and polyaromatic hydrocarbons. Furthermore, we determined the levels of 8-isoprostane in cord blood plasma samples in order to determine the degree of oxidative damage in newborns. Subsequently, we evaluated possible associations between POPs intake by the mother (along with diet quality) and birth weight and the degree of oxidative damage in newborns.

Results:

1. Dichlorodiphenyldichloroethylene (DDE) was the only substance present in all 352 daily food samples. DDE is a substance from the group of organochlorine pesticides. It is a metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT). Its occurrence in all samples is surprising, because the use of DDT has been prohibited in the Czech Republic since 1974.
2. The concentrations of most POPs in the diet were low and usually did not reach any established limits. The exception was perfluorinated compounds, whose permissible cumulative weekly intake was exceeded in four women.
3. The only group of POPs whose occurrence in food was higher in the area with a higher degree of air pollution was polyaromatic hydrocarbons.
4. Concentrations of polychlorinated biphenyls and organochlorine pesticides were higher in samples with higher amounts of fat, concentrations of DDT were higher in samples with higher amounts of dairy products, and concentrations of polyaromatic hydrocarbons were higher in samples with higher amounts of cereals.

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P 115 NUTRITIONAL AND ANTIOXIDANT POTENTIAL OF SOYBEAN (*Glycine max*) BY-PRODUCTS: AN EVALUATION STUDY

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Soybean (*Glycine max*) has played an important role in the human nutrition in the 21st century, not only through grain-based products, such as soybean oil, but also through its use in the production of vegetable beverages [1,2]. In light of pursuing healthier lifestyles and due to the growing demand for alternative beverages, soybean has emerged as a crucial ingredient in the development of a diverse range of plant-based beverages. The production of soybean beverages generates two key by-products of soybean processing, namely the okara and soybean hulls. However, these by-products are still potential sources of nutrients and bioactive compounds, thus can provide viable alternatives to traditional drinks. To assess the potential of soybean by-products and promote a circular economy by reintegrating by-products into the agri-food chain, the chemical and nutritional characterization of soybean hulls and okara was conducted. The proximal composition of the soybean by-products was determined using official AOAC methods. Analysis of free sugars, and organic acids was conducted using liquid chromatography with refractive index (HPLC-RI) and diode (UPLC-DAD) detectors, respectively. The fatty acid profile was assessed via gas chromatography with flame ionization detector (GC-FID), while total phenolic compounds were determined using the Folin-Ciocalteu method. Additionally, the antioxidant activity of the two extracts was evaluated through lipid peroxidation inhibition (TBARS), reducing power (RP), and 2,2-diphenyl-1picrylhydrazyl (DPPH) radical-scavenging activity assays. Regarding okara, this by-product exhibited high moisture content, with protein being the predominant macronutrient. The soybean hulls, on the other hand, demonstrated a predominance of carbohydrates and fiber, along with low moisture content. In terms of the lipid fraction, both by-products displayed a prevalence of unsaturated fatty acids, specifically oleic acid for okara and linoleic acid for soybean hulls. Oxalic acid was the only organic acid identified in okara, while citric acid was found to be predominant in soybean hulls. In relation to the total phenolic composition, the soybean hull exhibited higher values in mg of gallic acid equivalents (mg GAE/mL) compared to the okara sample. Both extracts demonstrated satisfactory antioxidant properties. These findings highlight that these by-products can be used as an interesting source of nutrients and potentially serving as sources of bioactive compounds, therefore offering opportunities for the development of novel functional ingredients in the food industry.

P 116 EXPLORING THE CHEMICAL AND NUTRITIONAL PROFILE OF MAIZE (*Zea mays* L.) CULTIVATED WITH BIOSTIMULANTS

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Considering the accelerated population growth and the increasing environmental pressures the planet is subject to, the effort to achieve a substantial increase in crop productivity within a production system with reduced environmental and social impact, not neglecting the nutritional needs of plants, has become more and more difficult. (1). Corn (*Zea mays* L.) is recognized as a prominent cereal cultivated globally, with significant economic importance attributed to its diversity of applications and uses. Consequently, to enhance the growth and yield potential of this crop, while minimizing environmental consequences, the utilization of biostimulants emerges as a viable alternative to conventional fertilizers. Biostimulants are categorized as bio-based agricultural products specifically designed to intensify crop development by fortifying plants against stress conditions and enhancing nutrient uptake capabilities (2). This study aimed to assess the impact of using microorganisms (*Metyloobacterium symbioticum*) as biostimulants in corn cultivation, specifically investigating their potential influence on the nutritional and chemical profile of the crop. The main objective was to ascertain whether their application could serve as a viable alternative to the utilization of chemical fertilizers. The nutritional composition was determined following official AOAC methodologies and encompassed moisture, ash, fat, proteins, carbohydrates, and energy. The chemical composition included soluble sugars (HPLC-RI) and fatty acids (GC-FID). The results showed that the minority components of corn are moisture (around 1.4± 0.1%) and ash (about 1.3 ± 0.2 g/100 g fw (fresh weight)). The control samples (without any type of biostimulant) showed a higher fat content (on average 2.7 ± 1.2 g/100 g fw) when compared to the samples treated with biostimulant (about 2.3 ± 1.1 g/100 g fw). On the other hand, the protein content showed higher values in the samples treated with biostimulants (7 ± 1 g/100 g fw). Concerning soluble sugars, it was evident the majority presence of sucrose, but it was also possible to identify the presence of fructose, glucose and raffinose. In general, polyunsaturated fatty acids showed higher percentages (around 47 ± 2 %), followed by monounsaturated fatty acids (33 ± 1 %), and lastly, saturated fatty acids (20 ± 1 %). Therefore, the study revealed that the tested biostimulants could increase protein and polyunsaturated fatty acids content in corn, making this crop a source of compounds of interest from a nutritional and bioactive perspective. In that way, the results seem promising; however, more bioactivity studies should be carried out in the future.

P 117 PRELIMINARY STUDY TO DEVELOP ECO-SUSTAINABLE FUNCTIONAL BEVERAGES FROM COFFEE PULP INFUSIONS

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The food industry is currently facing new challenges in designing eco-sustainable functional foods, beverages being the most widely accepted due to the ease of logistics and distribution, as well as the simplicity of bioactive compound integration. Phenolic compounds are one of the most attractive functional ingredients due to their antioxidant and anti-inflammatory properties, and their use from food by-products in beverage fortification has gained much attention since it contributes to both health and circular economy. Coffee pulp (CP), the main by-product discarded during the wet processing of coffee, representing 29% of the dry weight of the whole coffee cherry, is rich in dietary fiber and bioactive compounds. Recently, the EU has declared CP (referred to husk) as a novel food and safe ingredient for the preparation of soft drinks and infusions under EU Regulation 2015/2283. The aim of this work is to increase the knowledge about the potential antioxidant capacity of CP infusions to develop functional beverages of interest for the industry. Infusions were obtained from CP considering 2–3 mm of particle size, 0.05 CP/ water (g/mL) ratio and three extraction times (3, 6 and 9 min). Total phenolic compounds (TPC) were determined by Folin-Ciocalteu method and antioxidant capacity by ABTS procedure. The profile of phenolic compounds and caffeine were quantified by UHPLC-UV-VIS-MS. CP infusions presented a high TPC content (71–102 mg GAE/ 100 mL) and antioxidant capacity (125–185 mg TE/100 mL), which are in the range of herbal, coffee, and tea infusions (50–200 mg/ 100 mL beverage). Protocatechuic (6–11 mg / 100 mL) and chlorogenic (4.5–9 mg/ 100 mL) acids were the main phenolic compounds detected. The caffeine content was higher (22–39 mg/ 100 mL) than the individual phenolic compounds. Interestingly, all the compounds increased with the infusion time. Data suggest that bioactive compounds are affected by the extraction time, being responsible for bioactive compounds' solubility. These results are promising for the utilization of CP infusion to develop a functional beverage. However, other parameters like the particle size and the CP/water ratio could also affect the bioactive composition; thus, a multivariate optimization should be accomplished to know the best conditions to obtain a CP infusion with the highest antioxidant capacity.

P 118 A PROBIOTIC FUNCTIONAL FOOD ENRICHED IN PHYTOSTEROLS AND CAROTENOIDS PREVENTS METABOLIC SYNDROME WITH HISTOLOGICAL CHANGES IN ADIPOSE AND LIVER TISSUES IN HFD RAT

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Fermented vegetables food can be considered as functional food to prevent obesity and metabolic disorders associated[1]. A vegan fermented product, based on fermented maize and fruits, was designed to be functional, namely probiotic and enriched in papaya/melon carotenoids and dispersible phytosterols in order to obtain in addition a cholesterol-lowering effect. The objective of the study was to evaluate the effect of this new functional food on a HFD (High-Fat-Diet)-induced metabolic syndrome (MetS) rat model focusing on lipid metabolic disorders (body weight gain and fat mass, dyslipidemia in plasma and histological changes in fat and liver tissues).

Male Sprague-Dawley rats were randomly divided into 4 groups (n=9): a control group; an HFD group; Two HFD groups receiving 1 g/rat/day of the functional fermented food (maize 5% and fruits 30%) during two or three months following a curative or preventive mode. After enthanasia (isoflurane 4 %), blood, liver and fat mass were collected, weighted and analysed for biochemical and histology (Light microscopy 10X-BA-PAS and HES staining). Adipocyte and hepatic lipid droplets area determined by Image J software (One-way Anova followed by Tukey (HSD) post hoc test $p<0,0001$).

The HFD rats treated with the functional food showed a 20% lower weight gain compared to HFD rats. The preventive group was significantly different to the HFD group ($p<0.05$) and was similar to the control. Similarly, the percentage of adipose tissues relative to total body mass decreased by 22 % in rats treated with the functional product compared to the HFD group. Histological analysis of epididymal tissue showed an hypertrophy of adipocyte area of HFD rats (9801 µm2 vs. 5766 µm2 in control group), that was prevented by comsumption of the functional food. Interestingly, the histological liver analysis completed these observations showing a decrease of lipid droplet areas in rats fed with the functional food particularly for the preventive group (7.11 vs 5.75 µm2 respectively for HFD and HFD preventive group). Finally the functional food exerted significant anti-MetS effects by reducing LDL cholesterol and triglycerides (around 1.5 times compared to the HFD group).

These results validate *in vivo* the anti-MetS effects of this new functional food, associated to lipid metabolism, that could preventatively contribute to fight against MetS as an alternative to dairy products in both northern and southern countries.

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P 119 BIOACTIVE FOOD ADDITIVES BASED ON CHOKEBERRY JUICE POLYPHENOLS AND OAT

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Background:

Chokeberry juice is abundant in bioactive compounds such as polyphenols that have proven health benefits. The consumption of chokeberry juice is limited due to its astringency, while juice processing may cause the degradation of some valuable compounds. The scientific community is struggling to develop new methodologies and processes that will ensure maximum protection of health-promoting compounds. Previous studies have shown that polyphenols can be attached to various carriers (polysaccharides, proteins, etc.) and in this way preserve their properties. Freeze-drying is one of the most common techniques used to obtain such systems.

Objectives:

The aim of our research was to employ oat as a carrier of chokeberry juice polyphenols. Different percentages of oat (1%, 2%, 3%, 4% and 5%) were used to evaluate the impact of carrier amount on polyphenols adsorption. To ensure polyphenols adsorption and stable polyphenols-carrier samples, freeze-drying was applied. The antioxidant activity of such samples was evaluated as well as polyphenols content. Structural changes were proven by infrared spectra.

Methods:

The obtained samples were evaluated spectrophotometrically on the total polyphenols, anthocyanins and proanthocyanidin contents. Antioxidant activity was tested with four assays: FRAP, CUPRAC, ABTS and DPPH. For the identification and quantification of individual anthocyanins, HPLC analysis was used. The amounts of other polyphenols were determined by LC-MS/MS analysis. FTIR-ATR analysis was performed to observe structural changes.

Results:

The sample with 1% of oat had the highest content of total polyphenols, anthocyanins and proanthocyanidins as well as antioxidant activity. Anthocyanins present in samples were cyanidin 3-galactoside and cyanidin 3-arabinoside and their concentration decreased as higher percentages of oat were used. Furthermore, proanthocyanidins were determined in higher concentrations when higher amount of oat were used for complexation so the highest concentration of procyanidin B2 and procyanidin C1 were found in the sample with 5% of oat. The chlorogenic and neochlorogenic acids were dominant polyphenols in juice as well as in the samples.

Conclusion:

It can be concluded that prepared bioactive food additives had strong antioxidant activity due to the presence of chokeberry juice polyphenols. Depending on the amount of carrier, individual polyphenols will be adsorbed differently so analytical measurements are obligatory during preparation of food additives. Generally, the sample with 1% of oat possessed the highest concentration of polyphenols and antioxidant activity. Such additives can be used for fortification of different food products such as baked goods, smoothies, or yogurts. Future investigations will include applications of these additives in real food systems.

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P 120 ENHANCED GROWTH AND PRODUCTIVITY OF USEFUL METABOLITES IN THE *IN VITRO* CULTURE OF *Spirodela polyrhiza* (GIANT DUCKWEED) BY MELATONIN TREATMENT

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In this presentation, the effect of melatonin treatment on growth and useful metabolites production in *Spirodela polyrhiza* (Araceae family, giant duckweed) culture will be presented. *S. polyrhiza* is a duckweed species that serves as a potential resource for feed, food, bioremediation, and pharmaceutical applications. In this study, we assessed the effects of different concentrations of melatonin (0, 0.1, 1, and 10 μM) on the growth of *S. polyrhiza* during *in vitro* culture and the metabolic profiles and productivities of useful metabolites using gas chromatography–mass spectrometry coupled with multivariable statistical analysis. We found that exogenous melatonin significantly improved the total dry weight and altered the metabolic profiles of *S. polyrhiza* cultures. Melatonin significantly enhanced the cellular production of useful metabolites, such as γ-aminobutyric acid, dopamine, threonine, valine, and phytosterols. The volumetric productivities (mg/L) of γ-aminobutyric acid, dopamine, campesterol, β-sitosterol, and stigmasterol were the highest in the presence of 10 μM melatonin on day 12. Moreover, the productivities of ascorbic acid and serotonin were the highest in the presence of 1 μM melatonin on day 12. Therefore, melatonin could be used to enhance the production of biomass and useful metabolites during large-scale *S. polyrhiza* cultivation in cosmetic, food/feed, and pharmaceutical industries.

P 121 INCORPORATION OF OAT IN CHOKEBERRY/CARBOXYMETHYLCELLULOSE HYDROGELS: LC-MS/MS AND HPLC-DAD ANALYSIS

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Background:

Polyphenols are unstable components that are easily decomposed by heat, light, oxygen and other extreme conditions often applied during food processing. Their stability is also influenced by interactions with other components in the food matrix. The food industry is investigating new formulations that could ensure the protection of polyphenols from undesirable external effects. Hydrogels are a type of delivery systems that are consisted of polymeric materials with the capability to deliver polyphenols. Chokeberries are one of the richest sources of polyphenols that have antioxidant and anti-inflammatory properties, but their consumption is limited due to their pungent taste. The health benefits of oats have been attributed to the presence of fibers and polyphenols. Both chokeberry and oat offer many opportunities for future functional food development.

Objectives:

The aim of this study was to prepare carboxymethylcellulose hydrogels (1% and 1.5%) from chokeberry juice and the addition of oat (5% and 10%) to investigate its effect on total polyphenol, proanthocyanidin and anthocyanins contents and antioxidant activity as well.

Methods:

The total polyphenol, proanthocyanidin and anthocyanins contents and antioxidant activity of chokeberry/carboxymethylcellulose hydrogels using DPPH, ABTS, FRAP and CUPRAC assays were evaluated spectrophotometrically. The concentration of individual polyphenols incorporated in hydrogels was determined by LC-MS/MS analysis, while the concentration of 2 anthocyanins (cyanidin-3-galactoside and cyanidin-3-arabinoside) was determined using HPLC-DAD analysis.

Results:

The highest total polyphenol and proanthocyanidin content were detected for hydrogels prepared with 1% and 1.5% carboxymethylcellulose, while samples with oat addition had lower concentrations of both total polyphenols and proanthocyanidins. The same trend occurred for the determination of antioxidant activity. In all samples, neochlorogenic and chlorogenic acid were predominant components among other individual polyphenols identified and quantified using LC-MS/MS analysis.

Conclusion:

It was concluded that to accomplish higher retention of polyphenols in products, adequate formulation of the food systems is required. Prepared hydrogels could be used in the bakery and confectionery industry to improve the nutritional value of the products. Future research on the health benefits of oat and chokeberry for formulating novel food products is needed. This may increase the range of functional foods with beneficial health effects and the prevention of chronic diseases.

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P 122 HEALTH BENEFITS OF INNOVATED *Curcuma longa* EXTRACT

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Used traditionally for thousands of years in Asia, *Curcuma longa* is now of growing interest for its various health benefits in conventional medicine, cosmetology and nutraceutical. Indeed, turmeric's compounds, particularly curcuminoids, have already been shown to be active in anti-inflammatory, antioxidant, antibacterial, anticancer and hepato-protective effects. Their interest in the support of chronic pathologies is illustrated in several clinical trials. In addition, emerging studies show the biotransformation of turmeric improves health effects. It is with this objective that a new ingredient will be developed. The purpose of this work is the demonstration of increased bioactivities of an extract of *Curcuma longa*. Indeed, the final product will be distinguished by a plant totum obtained after an innovative biotechnology process. After the characterization of turmeric's bio compounds, health effects are demonstrated according to several study models such as an *in vitro* cellular model of intestinal barrier including Caco-2, HT29-MTX and U937 cells. The antioxidant mechanisms are studied according to 4 different tests: FRAP, DPPH, ABTS and Cuprac. Impact of digestion is also studied on the final product according to an *in vitro* model mimicking the gastrointestinal digestion. Finally, the health potential of this nutraceutical will be enhanced by optimizing the biotransformation process of turmeric.

Keywords:

Turmeric; Nutraceutical; Innovative; Bioactivities; Biotransformation

P 123 DEVELOPMENT AND CHARACTERIZATION OF A NOVEL FUNCTIONAL SPORTS DRINK BASED ON THERMAL WATER, HIBISCUS TEA, AND APPLE JUICE

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People are increasingly inclined to have an active lifestyle by exercising regularly. This has encouraged the consumption of sport-related products, such as sports drinks (SD). SD aim to supply energy and electrolytes in the form of quickly absorbable carbohydrates and minerals, mainly sodium. Recently, the sector of sports drinks has advanced towards the use of novel and functional ingredients intended to provide health-promoting effects beyond the basic functions of SD. In this context, the richness of minerals in thermal water (TW), including sodium, makes it a promising ingredient for the production of SD. The aim of this study was to develop an innovative functional SD based on TW (source of minerals), hibiscus (*Hibiscus sabdariffa*) tea (HbT, providing color and bioactive compounds), and apple juice (as a source of sugars and bioactive compounds). In the first step of the SD development, HbT from tea bags was prepared using TW with 0.1% citric acid, and a 2^2 central composite design was used to study the effect of time (30–120 minutes) and solid-to-liquid ratio (S/L, 1:10 – 1:150 m/v) on the content of total anthocyanins (TA) and total phenolic compounds (TPC). The results revealed that time was not significant for the extraction, while higher S/L yielded higher TA and TPC. The final conditions of preparation of HbT were S/L of 1:30 in 43 min. Then, the SD formulation was defined based on sensory characteristics (taste and visual color), and chemical and physico-chemical markers (content of sugars, soluble solids, pH, and sodium content). It was composed as follows (in 100 mL): 22 mL of HbT, 33 mL of TW, and 45 mL of apple juice. The SD was characterized for pH, soluble solids (SS °Brix), color (Cie L*a*b*), TPC, TA, anthocyanins, and non-anthocyanin phenolic compounds by LC-DAD-ESI-MS^n, sugars by HPLC-RI, and sodium content by atomic absorption spectrometry. The antioxidant activity was also evaluated by ORAC (Oxygen Radical Absorbance Capacity) and a cell-based assay using RAW 264.7 cell line. The anti-inflammatory activity of SD was determined using RAW 264.7 cell line. The pH was 3.72 ± 0.003, SS was 5.67 ± 0.05 °Brix. Color was L=54.51, a*=34.48, b*= 21.77. The SD had fructose, glucose, and sucrose, yielding the levels of total sugars usually present in SD (6.9 ± 0.11 g/100 mL total sugars). The same can be stated for the sodium content (33 mg/100 mL). TPC and TA were 40.68 ± 1.18 mg GAE/100 mL and 4.08 ± 0.07 mg cyanidin 3-glucoside equivalent/100 mL. As expected, the main anthocyanins were delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside. Concerning the non-anthocyanin phenolic compounds, the results showed that SD has mainly hydroxycinnamic acid derivatives, such as caffeoylquinic acid isomers and *p*-coumaroylquinic acid. For antioxidant and anti-inflammatory activities, the results revealed that the SD showed promising bioactivities, suggesting that the beverage has proven to be an interesting alternative for physical activity practitioners.

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P 124 *Prunus* SPP. BY-PRODUCTS UTILIZATION: BIOACTIVE COMPOUNDS AND TECHNOLOGICAL APPLICATIONS

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Fruits and vegetables are among the most important sources of phytochemicals worldwide. A lot of attention is currently being paid to the valorization of fruit by-products, and their application in various industries. The ongoing growing demand for food poses important environmental challenges. The genus *Prunus* includes fruits that are highly preferred for consumption, and as such, generate large amounts of by-products and waste. It has been documented that by-products can be nutritious, and excellent functional ingredients not exclusively to the food industry.

In the current review, we aim at summarizing the most valuable bioactive compounds in this species, and their implementation in food as highly-valued supplements in the view of sustainability and eco-friendliness. Pomace, kernels, peels, and leaves represent valuable sources of polyphenols, unsaturated fatty acids, carotenes, tocopherols, sterols, squalene, and proteins (incl. peptides and amino acids). The spectre of bioactivities will also be discussed. Possible ways for implementation in food products will also be presented. Recommendations and future perspectives in the view of efficient waste management will be evaluated for better resource utilization.

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P 125 THE EFFECTS OF 12-WEEK MULTIVITAMIN SUPPLEMENTATION ON EVERYDAY FUNCTION IN OLDER ADULTS: A RANDOMISED, PLACEBO-CONTROLLED TRIAL

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Background:

Research has indicated the potential for multivitamin-mineral (MVM) supplementation to reduce depression, anxiety and stress and improve aspects of mood and cognitive function in older adults. However, this research rarely assesses outcomes that are pertinent to the daily lives of older adults.

Objectives:

The study had three main objectives, the first T to investigate the effectiveness of a MVM supplement on meaningful outcomes of everyday functioning in older adults. Second, to test this efficacy in males and females separately. Finally, the study aimed to widen inclusion criteria compared to previous trials, to be more representative of older adults.

Methods:

This randomised, double-blind, placebo-controlled, parallel groups trial investigated the effect of 12-week MVM supplementation on a sample of 228 adults, aged 70 and over. Outcomes relating to wellbeing, mood, and memory; physical health and activity; and social interaction and loneliness were measured at baseline and after 12 weeks.

Results:

Data was split by sex and analysed using one-way independent groups ANCOVA, controlling for scores at baseline. MVM supplementation led to increased feelings of friendliness in females (p=0.045). In males, following MVM, there were lower levels of prolonged stress reactivity (p=0.007), lower overall stress reactivity (p=0.019), and lower emotional loneliness (p=0.042).

Conclusion:

These results provide further support for the beneficial effects of MVM supplementation in older adults. Moreover, as outcomes are more pertinent to aspects of daily functioning in older adults, these findings provide a more accurate representation of how these supplements may affect individuals in their everyday life.

P 126 INCORPORATION OF CURCUMIN-LOADED SOLID LIPID NANOPARTICLES IN YOGURT: EVALUATION OF CURCUMIN'S BIOACCESSIBILITY AND *IN VITRO* CYTOTOXICITY

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One of the main objectives of food researchers and producers is to develop and manufacture functional foods with improved nutritional and health properties due to the increasing consumer concern for healthier lifestyle. Curcumin is a lipophilic nutraceutical with several health-promoting properties. However, it has poor solubility and it is quickly degraded on aqueous solution, hindering its application into food products. Encapsulation of curcumin in lipid-based nanostructures, such as solid lipid nanostructures (SLN), is one of the strategies to improve its stability and bioavailability.

The main goal of this work was to evaluate the effects of dynamic *in vitro* digestion on curcumin-loaded SLN incorporated in a yogurt (SLN-yogurt). Curcumin's bioaccessibility and stability, and protein's hydrolysis degree (HD) were evaluated during *in vitro* digestion. Furthermore, the effect of the digested samples on Caco-2 cell viability was assessed.

SLN-yogurt and control 1 (i.e., yogurt with no added SLN) presented an increase of the protein HD over digestion showing the highest HD values at the end of duodenum, 31.4 % and 56.6 %, respectively. Regarding to curcumin's bioaccessibility and stability, both control 2 (i.e., free SLN) and SLN-yogurt presented similar values either for curcumin's bioaccessibility and stability, showing that the yogurt matrix did not affect significantly the curcumin's fate at the end of digestion. Finally, both controls (1 and 2) and samples were subjected to a cell viability test after digestion, where both controls (1 and 2) showed less negative effect on cell viability than SLN-yogurt. This result could be due to the digestion products formed by the presence of SLN and yogurt ingredients during digestion which can decrease the cell viability.

In general, the presence of SLN within yogurt decrease the protein HD but did not affect the curcumin's bioaccessibility and stability. However, SLN-yogurt decreased cell viability more than SLN and yogurt analyzed separately. Despite cell viability reduction when in contact with SLN-yogurt after *in vitro* digestion, SLN alone did not affect cell viability. In conclusion, our results suggest that SLN has a high potential to be used as delivery system in a functional food as a strategy to increase the nutraceuticals' bioaccessibility.

P 127 1'-ACETOXYCHAVIOL ACETATE FROM *Alpinia galanga*: A PROMISING ANTI-INFLAMMATORY AGENT FOR FUNCTIONAL FOOD SUPPLEMENTS

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Background:

Functional foods and food supplements have emerged as a promising approaches to promote health and prevent diseases. Inflammation is a critical process that defends the body against infections but can also contribute to the development of chronic diseases such as cardiovascular disease, cancer, and diabetes. Inflammation is a process in which the body's immune system protects the body from viruses and bacteria by utilizing various mechanisms. Inflammation can be acute or chronic, thus it would require different mechanisms to restore the body to its normal function and state.

Objectives:

The aim of this study is to determine the anti-inflammatory activities of 1'-acetoxychaviol acetate in comparison to Indomethacin.

Methods:

In this study, pure 1'-acetoxychaviol acetate which was extracted from *Alpinia galanga* L. was used to observe their effectiveness as a medicine against inflammation. The pure extract of 1'-acetoxychaviol acetate was evaluated for the anti-inflammatory activities via monitoring the nitric oxide production, along with checking the MTT assay to check for its toxicity to mammalian cells. In this study, 1'-acetoxychaviol acetate was tested alongside Indomethacin on the RAW264.7 murine macrophage. The results indicated that the pure extract of 1'-acetoxychaviol acetate has better anti-inflammatory activities than the Indomethacin.

Results:

The 1'-acetoxychaviol acetate has an IC_{50} of 7.7 µg/mL which was significantly lower than Indomethacin which has an IC_{50} of 39.8 µg/mL. However, the 1'-acetoxychaviol acetate becomes toxic at a higher concentration as shown by the cell viability. The results validate the possible uses of 1'-acetoxychaviol acetate as an anti-inflammatory medicine – however, due to its toxicity at a higher level, it would be best to use with caution. In conclusion, this study provides evidence for the anti-inflammatory properties of 1'-acetoxychaviol acetate extracted from *A. galanga*.

Conclusion:

The findings suggest that this natural compound could be developed into a functional food supplement to promote health and prevent chronic diseases. Further research is needed to validate the efficacy and safety of 1'-acetoxychaviol acetate as a food supplement, which could pave the way for the development of novel functional foods with anti-inflammatory properties.

Keyword:

1'-acetoxychaviol acetate; Alpinia galanga; Nitric oxide; Medicinal plants; Anti-inflammation

P 128 EXPLORING THE ANTI-INFLAMMATORY PROPERTIES OF *Curcuma longa* EXTRACT: IMPLICATIONS FOR FUNCTIONAL FOODS AND FOOD SUPPLEMENTS

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Background:

Functional foods and food supplements have gained significant attention in recent years due to their potential to promote health and prevent diseases. Natural compounds, such as *Curcuma longa* extract, have emerged as a safer and more sustainable alternative to conventional anti-inflammatory drugs. However, further research is required to determine the optimal dosage and long-term safety of *Curcuma longa* extract as a food supplement. The objective of this study was to investigate the anti-inflammatory effects of *Curcuma longa* extract.

Methods:

The production of nitric oxide was measured to determine the anti-inflammatory effects of *C. longa* extract. Additionally, an MTT assay was performed to assess the extract›s toxicity levels towards mammalian cells.

Results:

The study found that *C. longa* extract has better anti-inflammatory activities than Indomethacin, a commonly used anti-inflammatory drug. The *C. longa* extract exhibited an IC50 of 10.80 µg/mL, significantly lower than Indomethacin›s IC50 of 36.20 µg/mL. Furthermore, the *C. longa* extract demonstrated no toxicity towards RAW264.7 cells up to a concentration of 50 µg/mL.

Conclusion:

The study highlights the potential of *C. longa* extract as a functional food supplement with anti-inflammatory properties. The development of food supplements with anti-inflammatory properties is of significant interest to researchers and consumers. Further research is required to determine the optimal dosage and long-term safety of *Curcuma longa* extract as a food supplement.

Keywords:

Functional foods; *Curcuma longa* extract; Anti-inflammatory properties; Natural compounds; Nitric oxide

P 129 ANTIOXIDANT AND IMMUNE ENHANCING EFFECTS OF BLACK GINSENG FERMENTED THROUGH SACCARIFICATION WITH MALT IN THE RAW264.7 CELLS AND IMMUNE-DEPRESSED C57BL/6 MICE

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The aim of this study was to investigate the potential antioxidant and immune-enhancing effects of black ginseng fermented through saccharification with malt using *in vitro* and *in vivo* models. The functional activities of fermented black ginseng (FBG) were assessed by evaluating its antioxidant and immune-enhancing effects. The results showed that FBG exhibited high DPPH and ABTS radical scavenging activities, along with xanthine oxidase (XO) inhibitory activity, which increased in a dose-dependent manner. FBG also demonstrated increased superoxide dismutase and catalase activities in RAW 264.7 cells, with no observed cytotoxicity. Additionally, nitric oxide and cytokine concentrations (IL-1, IL-6, IFN-γ, and TNF-α) in RAW264.7 cells significantly increased in a dose-dependent manner. In immunosuppressed mice induced by cyclophosphamide, FBG50 (50 mg/kg body weight) and FBG100 (100 mg/kg body weight) groups exhibited higher white blood cell count and B cell proliferation of splenocytes compared to the negative control (NC) group. Serum IgG and cytokine (IL-1 and IFN-γ) levels were also higher in FBG50 and FBG100 groups compared to the NC group, and FBG treatment increased NK cell activity compared to the NC group. Overall, these findings suggest that FBG has the potential to improve antioxidant and immune stimulating effects, making it a promising and innovative therapeutic approach for managing related conditions and promoting public health.

P 130 WILD BLUEBERRY EXTRACT INTERVENTION IN HEALTHY OLDER ADULTS: A MULTI-STUDY INVESTIGATION OF ACUTE COGNITIVE AND CARDIOVASCULAR BENEFITS

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Background:

Common symptoms of age-related cognitive decline are impaired executive functioning (EF) and episodic memory (EM) deficits, both of which are also age-sensitive and a common hallmark of dementia. Existing evidence shows that anthocyanin-rich foods such as berries may improve, or attenuate decline in EF and EM domains in ageing adults. Previously, chronic supplementation with daily wild blueberry extract (WBE) has improved episodic memory and reduced systolic blood pressure.

Circadian and homeostatic declines in cognitive performance are observed during the day, most commonly at 14:00. Additionally, a post-prandial reduction in cognitive ability has been demonstrated 1 h after lunch consumption, affecting domains of EF, memory and attention. Further research is required to assess whether extracts such as WBE may be beneficial for cognitive function across an acute timeframe including known periods of reduced functioning.

Objectives:

1) Study 1: ROAB: To investigate the efficacy of WBE in maintaining EF and EM throughout the day alongside measures of cardiovascular outcomes in healthy older adults. A range of WBE doses were utilised to identify the optimal dose at which cognitive and cardiovascular effects occur. **2) Study 2: BEAT:** The aim of Study 2 was to replicate alleviation of cognitive decline during a predicted post-lunch dip whilst also improving cardiovascular outcomes following acute WBE 222 mg supplementation.

Methods:

Both studies employed a randomised, double-blind, cross-over, placebo-controlled design to explore the effects of WBE intervention versus placebo on several outcomes, including EM, EF, blood pressure and heart rate in a healthy older adult population (aged 68–75). In ROAB, 28 participants received WBE 111 mg, 222 mg, 444 mg, 888 mg and placebo over a 5-week period separated by a 1-week washout. Outcomes were measured at 0 h, 2 h, 4 h and 6 h post-intervention with intervention occurring immediately after baseline (0 h). In BEAT, 45 participants received WBE 222 mg and placebo (1-week washout). Outcomes were measured at 0 h and 6 h (14:00) when a post-lunch dip was anticipated. This was further enhanced by consumption of lunch 1 h prior to cognitive testing. The WBE 222 mg intervention aligned with known peaks in plasma blueberry polyphenol metabolites at 2 h which would coincide with a predicted drop in post-lunch performance.

Results:

ROAB: A significant dip in executive function was apparent at the 4 h timepoint for placebo only, indicating attenuation for WBE doses. Strikingly, WBE 222 mg produced acute reductions in both systolic and diastolic blood pressure compared with placebo. **BEAT:** EF reaction time was found to be significantly faster for WBE 222 compared to placebo at the predicted post-lunch dip (14:00), with no other notable benefits on a range of cognitive and cardiovascular outcomes.

Conclusion:

These two studies indicate that wild blueberry extract may have cardiovascular benefits and attenuate natural cognitive decline observed over the course of day, particularly when a decline is associated with a circadian rhythm driven post-prandial dip. However, it is important to acknowledge that effects were subtle and benefits were only observed on small number of outcomes. Further research is required to explore the utility of WBE in populations already experiencing mild cognitive impairments.

P 131 PRODUCTION AND EVALUATION OF A NEW ALTERNATIVE PROTEIN WITH PREBIOTIC ACTIVITY AND A POTENTIAL SOURCE OF BIOACTIVE THROUGH A BIOCONVERSION PROCESS OF CHILEAN BROWN SEAWEED *Durvillaea* SPP. AND AN ARTIFICIAL CONSORTIUM OF FOOD FUNGI

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The production of animal-origin proteins to satisfy human needs require abundant natural resources and also their intake is associated with health problems. In this research, we obtained for the first time an alternative and functional protein food rich in proteins, ß-D-glucans, and contains various metabolites, including bioactive sugars, organic acids, and phenolic compounds. This product is the result of enhancing the nutritional value of the Chilean brown seaweed *Durvillaea* spp. through a fermentation process with a unique artificial consortium of marine and terrestrial fungi. This is regarded as part of the solution to debottleneck the physiological limitations of pure cultures bioprocesses, such as degrading complex carbon sources and efficient substrate utilization. This rationally designed consortium demonstrated properties that exceeded the mono culture properties, including a ~150% increase in productivity, an increase in total protein (~336%), amino acids (~245%), ß-D-glucans (100%) and total (Poly)phenols (75%). This protein known as mycoprotein has all the essential amino acids, low content of fatty acids, high level of dietary fibre (35%), high antioxidant activity (TEAC of 34 µM/g), good concentration of (Poly)phenols (1,4 mg/g EAG), without toxic metabolites, heavy metals, and pesticides. The nutritional composition of this food was evaluated, obtaining a yield of 561.3 g of mycoprotein from 1 kg of dry seaweed, a good concentration of total protein (35 g/100 g), a considerable concentration of total amino acids (21 g/100 g), a good concentration of ß-D-glucans (22 g/100 g). Additionally, the functional properties of this mycoprotein were evaluated. The properties to be considered were: antioxidant (radical eliminator DPPH and ABTS), anti-obesity, and anti-diabetic. Inhibition of pancreatic lipase, α-glucosidase, and α-amylase enzymes was used to determine anti-obesity and anti-hyperglycemic activity. In this study, an untargeted metabolomics approach combined with multivariate statistical analysis and dereplication techniques aided by the GNPS Molecular Network was employed to screen the metabolites and to identify molecules with nutraceutical properties. Among the screened 110-top differential metabolites, 22 nutraceutical compounds showed higher content in co-culture. In conclusion, the alternative protein from an artificial fungi consortium through a process of submerged fermentation with *Durvillaea* spp. as sole carbon source results in a mycoprotein that has a high-quality protein, great nutritional value, with prebiotic and nutraceutical potential. Also, we found an increase in the diversity of mycochemical compounds which represents a new source of drugs, nutraceuticals, and functional food.

P 132 ANTIOXIDANT POTENTIAL IN CELLULAR MODELS OF PHENOLIC EXTRACTS FROM INDUSTRIAL WASTE OF PEANUT PROCESSING

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Peanut (*Arachis hipogaea* L.) is a leguminous plant that is widely consumed worldwide and, in addition to having a high nutritional value, has several bioactive compounds in its composition, especially in its skin, which is a residue discarded in several industrial processes of in the food industry. The use of this raw material for the production of functional ingredients would follow the current trend of up-cycling, combining the possibility of generating value with the sustainable disposal of this waste, to the addition of nutritional quality in other food products that add these bioactive compounds as functional ingredient. With these objectives in mind, this study aimed to evaluate some parameters of the hydroethanolic extraction of phenolic compounds from the industrial residue of peanut skin, as well as to evaluate the antioxidant capacity of these extracts in *in vitro* models. A Rotational Central Composite Design (DCCR) 23, with 3 replications at the central point, was proposed to study the influence of the variables 1) ethanol concentration on the hydroalcoholic extractor solution, and 2) proportion of solid sample (waste) per liquid in the extraction (m /v) in a simple solid: liquid extraction process. The lowest possible ethanol content was aimed to remain an environmentally friendly and with low toxicity extract. The responses evaluated in the DCCR were: total phenolic concentration and antioxidant capacity by the ORAC method. According to the results obtained through the statistical analysis of the data, the concentration of total phenolics and the antioxidant capacity of the extracts obtained from the initial extraction conditions, to the final ones of the study increased significantly – ranging from 215.15±4.79 to 537 .87±3.94 mg AGE/g extract and from 1200.20±84 to 3163.37±318 µmol eq. Trolox/g extract, respectively. The optimal extraction conditions within this model was: 50% ethanol in water, and the proportion of sample: extraction solution (mass/volume) equal to 0.2. The obtained extract presented very high ORAC rates comparing to similar residue extracts on the literature. A cellular model with human intestinal epithelium cells (Caco-2) was used to evaluate the ability to modulate intracellular reactive oxygen species (ROS). The experimental cellular model an oxidation-sensitive fluorescent probe and AAPH (50 umol/L) as free radical to challenge the cells. While AAPH increased ROS production in cells by 240% compared to the negative control (cells without any intervention), the concomitant addition of the AAPH and extracts, completely neutralized its action, reducing the ROS production by the cells to the same content in the negative control cells. Conclusion: The mass ratio of peanut skin residue and hydroethanolic solution was optimized to obtain phenolic extracts with higher phenolic contents and ORAC values, with the lowest use of organic solvent (ethanol). The extract obtained has high antioxidant capacity, both in chemical models (ORAC), as in cellular models, with great potential for scavenging free radicals. This extract is a potential functional ingredient for the food industry, coming from low cost raw material, wide availability and high bioactive potential.

P 133 EVALUATION OF THE EFFECTS OF DIFFERENT PRODUCTS DERIVED FROM THE BLACKBERRY PLANT IN A RAT MODEL OF CHRONIC STRESS

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Background:

The neuroprotective effects of berries are largely attributed to their high content of phytochemical compounds. Blackberries are particularly rich in flavonoids, especially anthocyanins, which can cross the blood-brain barrier and exert neuromodulatory effects. These compounds exhibit protective effects against oxidative stress and inflammation, which play significant roles in the development of mood disorders such as anxiety and depression. Therefore, polyphenols have been proposed as a novel approach for the prevention or treatment of these conditions.

Objectives:

The main aim of the present study was to evaluate whether chronic consumption of blackberries (Berryum Nemus variety), both fresh fruit and a polyphenol extract of this fruit, could contribute to an improvement in the mood state of animals by reducing anxious response in a rat model after the exposure to the unpredictable chronic mild stress (UCMS) protocol.

Methods:

50 male Sprague-Dawley rats were randomly distributed in two different cohorts (n=10 control, n=40 UCMS). The UCMS protocol consisted in a daily application of two randomly selected stressful stimuli. UCMS-exposed animals were divided in four different groups depending on the received treatment: UCMS animals receiving control diet (UCMS-Control), UCMS receiving 10 g/kg/day fresh Berryum Nemus (UCMS-BN), UCMS receiving diet supplemented with 0.75% extract from Berryum Nemus (UCMS-Extract), and UCMS-animals receiving 15 mg/kg/day fluoxetine, a commercial antidepressant (UCMS-Fluoxetine). Animals were exposed to UCMS protocol and treatments for 29 days. At day 25, circulating corticosterone levels were analyzed after a physical restraint test. At day 29, animals were exposed to the elevated plus maze (EPM). Finally, at day 30, animals were sacrificed, and hippocampus were dissected for BDNF quantification (Figure 1A).

Results:

The percentage of distance travelled in the open arms of the EPM apparatus was significantly lower in UCMS-Control animals compared to Control animals. Interestingly, blackberry extract consumption partially reverted this anxious behavior, similarly to fluoxetine treatment (Figure 1B). Serum corticosterone levels after 30 min of physical restraint were significantly lower in UCMS-Extract and UCMS-Fluoxetine animals compared to UCMS-Control animals (Figure 1C), suggesting a reduction in the Hypothalamic-Pituitary-Adrenal axis (HPA) hyperactivity after a stress stimulus. However, fresh blackberries consumption was not effective in this model. Noteworthy, to achieve the daily polyphenol dose consumed by UCMS-Extract animals in this study, approximately 1200 g of fresh blackberries would be needed in a 60 kg human.

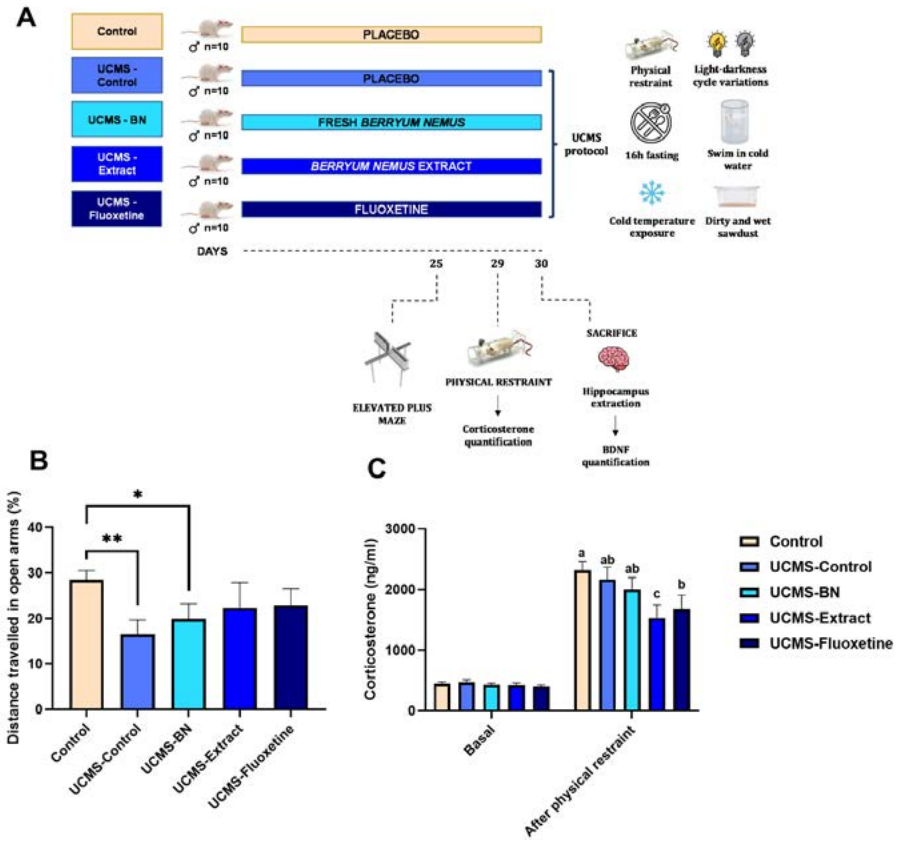

Figure 1: Experimental design scheme (**A**), percentage of distance travelled in the open arms of EPM (**B**) and serum corticosterone levels before and after 30 min physical restraint (**C**). * and **, p<0.05 and p<0.01. [abc], unlike letters mean significant differences among groups.

Conclusions:

The consumption of a blackberry polyphenol extract showed anxiolytic effects in rats exposed to chronic stress. This positive effect may be mediated by a downregulation in the HPA axis, as evidenced by the lower levels of corticosterone after stress induction. Moreover, the lack of favorable results from the consumption of fresh blackberries highlights the importance of dietary supplements containing concentrated polyphenol extracts to reach effective doses that would not be achieved with the daily consumption of fresh fruit.

P 134 FRUIT POMACE FLOURS: RECYCLING AGRI-FOOD FRACTIONS TO INNOVATIVE FUNCTIONAL FOODS

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Fruits and vegetables are responsible for about 22% of food losses and wastes along the supply chain. However, these by-products may be transformed into bioactives and fiber-rich flours and, thus bringing value to the food industry due to health benefits, nutritional properties, and technological functionality.

Fruit pomaces are relevant sources of macromolecular antioxidants, which are plant food macromolecules, such as polysaccharides or proteins associated with phenolic compounds. In the last years, there has been a considerable interest in understanding and exploiting the functions and health benefits of the macromolecular antioxidants, as they yield bioavailable metabolites during the digestion process, with significant effects either local and/or systemic after absorption, thus presenting an increasing interest in nutrition and health.

In this work, it is intended to use an apple pomace, a waste generated from the fruit juices production, to produce a dietary fibers and polyphenol' rich flour and to assess polyphenols potential bioaccessibility, through in *vitro studies*. Apple pomace flours were subjected to an *in vitro* simulated gastrointestinal digestion, according to INFOGEST method. The release of polyphenols and consequential bioaccessibility and carbohydrates' hydrolysis during digestion was investigated. The antioxidant activity of the bioaccessible fraction was also determined. The intestinal digests were subjected to several purification steps to avoid cell cytotoxicity (enzymatic inactivation, conductivity values and minimal dilution) and their absorption was quantified during a 2 h period using caco-2 barrier model.

The health potential of fruit pomace flours opens new opportunities for the exploitation of these agri-food wastes as a functional ingredient containing phenolic compounds, dietary fiber, and low glycemic index.

Acknowledgments:

Financial support is acknowledged to FCT through research contract (CEECIND/00029/2018/CP1545/CT0010 and CEECINST|00064|2021) and research unit LAQV-REQUIMTE (UIDB/50006/2020), through national funds and cofinanced by FEDER, under the Partnership Agreement PT2020. The authors gratefully acknowledge to all entities.

P 135 RANDOMISED DOUBLE-BLIND PLACEBO-CONTROLLED TRIAL TO INVESTIGATE THE IMPACT OF *Melissa officinalis* L. (LEMON BALM) ON SLEEP QUALITY AND MOOD IN AN ADULT POPULATION WITH MILD SLEEP DISTURBANCE

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Background:

Sleep is an essential component of physical and emotional well-being. Sleep problems emerging from lack of or disruption to sleep are associated with physical and emotional health concerns. Alongside pharmacological interventions, there are various over-the-counter herbal treatments (i.e. valerian, lemon balm, Californian poppy) to improve sleep which are increasing in popularity, possibly due to ease of consumption and absence of side effects. One such herbal is *Melissa officinalis L*. (lemon balm) which is rich in essential oils, triterpenoids and polyphenols including specific phenolic acids such as rosmarinic acid. Nevertheless, despite lemon balm demonstrating sleep quality improvement in various animal models, the evidence from good quality clinical trials capturing sleep changes with both objective (i.e., research-grade actiwatch, an alternative to polysomnography outside of a sleep laboratory) and subjective measures is limited. Here, we ran a randomised double-blind placebo-controlled parallel groups trial to examine the impact of 28 days of 300 mg-daily administration of lemon balm (or matched placebo) on sleep and mood.

Objectives:

The primary aim was to investigate objective and subjective sleep quality following 300 mg daily dose of lemon balm over 28-days in 99 young to middle-aged healthy adults with non-clinical mild-to-moderate sleep problems (defined as a Pittsburgh Sleep Quality Index score of ≥5). Secondary aims were to examine effects on transient mood measures assessed with standardised questionnaires, and effects on salivary cortisol, melatonin, and gut microbiota changes, which are biological measures involved in regulating circadian rhythms and known to be associated with sleep quality (i.e. sleep-wake patterns).

Methods:

Participants were randomised to receive either 300 mg lemon balm or matched placebo capsules every night before bed for 28-days. Participants were invited to attend the laboratory on 3 occasions, one familiarisation visit prior to treatment onset and two test visits on day 0 and day 28 of treatment. At each test visit, measures of sleep (PSQI, ISI, LSEQ) and mood (including PANAS-N, DASS-21) were completed. Stool and saliva samples were collected in the 24 h window before each visit. Sleep data was collected with a wrist-secured actiwatch worn over the 28-day treatment period (MotionWatch 8, CamNtech) in conjunction with a daily sleep diary. The study was registered on clinicaltrials.gov (identifier: NCT05422599).

Results:

Changes in sleep quality, measured by sleep onset latency and sleep efficiency, will be assessed alongside changes in transient mood, salivary analytes (melatonin and cortisol) and beneficial gut microbiota within multiple layers of an LMM. Data is currently being analysed and will be presented at the conference. The primary objectives are summed pre- and post-PSQI scores and components (i.e. sleep quality), and averaged time-bins (4-blocks of 7-days) of actiwatch-diary variables (i.e. fell asleep time, sleep efficiency). Secondary measures are clustered sleep/mood pre- and post-scores.

Conclusion:

Assessment of the effect of lemon balm to enhance sleep quality will likely improve confidence of individuals wishing to supplement lemon balm to resolve mild sleep disturbances, and contribute to an improved quality of life by reducing the onset of any physical and emotional health concerns associated with sleep deprivation.

P 136 DIVERSITY OF LIPIDS FROM ALGAE TO LAND PLANTS: LIPIDOMICS AS A PLATFORM TO ANALYSE THE POLAR LIPID MOLECULAR SPECIES EMERGING AS FUNCTIONAL INGREDIENTS

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Plant and algae are sources of omega-3 polyunsaturated fatty acids (PUFA), namely alpha-linolenic (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are mainly esterified in polar lipids (e.g., phospholipids and glycolipids). These lipids with the ability to regulate important biological functions are called beneficial functional lipids, being recognized not only for their nutritional value but also for their health-promoting properties including anti-inflammatory, antioxidant, or anti-microbial potential. But the polar lipidome of algae and plants seem to be species and/ or variety specific for the ones studied so far, yet to be fully unravelled for most of the species. It can also be chapped by distinct biotic and abiotic conditions, which can enhance the composition in most bioactive lipids. Therefore, the identification of their lipid signatures is essential to explore their nutritional and functional value to full explore their add-value as (functional) food and ingredients.

Lipidomic approaches based on liquid chromatography coupled to mass spectrometry (LC-MS) are currently being used to profile and identify the promising and valuable bioactive compounds, enabling a better understanding of their functional importance. In our studies, the analysis of the full polar lipidome of distinct algae, including macroalgae and microalgae species, and from avocado by-products (peel and seed) using high-resolution LC-MS allowed to characterize a wide variety of polar lipid molecular species belonging to glycolipids, phospholipids, sphingolipids, or betaine lipids, from which some were already reported with bioactivity. Moreover, it was also demonstrated that species with different composition in lipids showed different anti-inflammatory potential *in chemico* and in *in vitro* studies using macrophages. Therefore, the accurate identification of the lipidome of distinct plant and algae can contribute to enhance their valorisation fostering innovative biotechnological and industrial applications.

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P 137 FUNCTIONAL PROPERTIES OF GRAPE POMACE USED IN THE EXTRUSION PROCESS

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Grape pomace (GP), a winery industry by-product, could be transformed into extruded snacks. The consumption of these kind of foods has raised worldwide, representing a relevant part of diets. In order to effectively incorporate GP into extruded products it becomes important to know its functional properties since the processing involves the use of some type of physical stress. Moreover, functional properties influence the way each GP component interacts with the others from major raw materials in snacks. This knowledge is useful for setting the extrusion process parameters for different mixtures formulated to obtain the desired quality of the extrudate products.

The aim of this study was to determine the behavior during hydration in terms of water retention capacity (WRC) and swelling capacity (SC) of GP (seedless and with seeds), from two varieties (white and red), dehydrated by using two methods (dried in oven and lyophilization), and to evaluate if these different GP samples could be grouped based upon functional and chemical characteristics. The dried GP was ground into homogeneous powder by using a laboratory mill and sieved through 200 µm mesh.

The results revealed that the functional properties of GP are not only dependent on the grape variety, but also on their processing parameters and method used for GP preservation. Moreover, the structural properties of the GP flour, particle size and porosity can have a strong effect on the variation in WRC and SC. During GP lyophilization which is a low-temperature drying process, porous structures are easily developed within the cell wall matrix, and easy and complete rehydration is enabled, thus showing higher SC. Different WRC and SC values were exhibited by GP samples, seedless and with seeds, fact that can be explained by the variation in chemical composition. The variation of WRC and SC can be related to the insoluble polysaccharides from GP which can bind water by either surface tension in the pores of the matrix or ionic bonds, hydrogen bonds and/or hydrophilic interactions. The high WRC found for GP with seeds might be due to the insoluble fractions of fiber, such as cellulose, hemicellulose and lignin which tend to absorb water. Instead, since the WRC and SC are not relevant to soluble polysaccharides, it is possible that the soluble fiber in the total dietary fiber from GP seedless would contribute to the lower values. Principal component analysis results showed that the GP samples can be grouped, correlating functional and chemical features. These functional properties of GP and its composition will dictate the transformation of raw ingredients during extrusion processing and the final quality and stability of the extruded products.

Keywords:

Grape pomace; Drying methods; Hydration properties

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P 138 THE IMPACT OF TWO DIFFERENT DRYING METHODS ON THE POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF GRAPE POMACE

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Grape pomace (GP), the by-product generated in high amounts in the winemaking process, can be reused to produce novel foods due to its valuable components such as phenolic compounds, and natural antioxidants. Market demands for food products rich in phenolic compounds with many positive health benefits including antibacterial, antitumor, antioxidant, and anti-inflammatory effects. Wet GP is very sensitive to microbial spoilage and degradation of its bioactive components. Its high moisture and seasonality production require the application of fast and economical preservation methods that allow stabilization and storage for later use, and re-enter in the food cycle, thus avoiding environmental disturbances. Drying methods have a great impact on the stability of bioactive compounds, the phenolic content degradation being linked to the drying temperature-time combination.

In this study, the impact of two drying methods, convective oven drying (O) and lyophilization (L) on the antioxidant activity (AA) and total polyphenolic content (TPC) of grape pomace seedless (SGP) and with seeds (GP), from two grape varieties, white (W) and red (R), for potential application in functional foods like snacks have been investigated. A laboratory convective oven was used to oven drying of GP at 40 °C for 8 h, whereas a Biobase BK-FD12 lyophilizer was employed to carry out samples lyophilization, the freeze-dried process being performed at −50 °C over 24 h at a pressure of 10 Pa. The dried GP was then milled to pass a 200 µm screen. The total phenolic content and antioxidant activity were determined by spectrophotometric methods.

There was no significant ($p > 0.05$) difference in TPC among GPR_O and GPR_L, but the latter sample presented a significantly higher AA than that of GPR_O. Probably, the phenolic compounds with a high AA were better retained in GPR_L. The SGPR showed a TPC and AA much higher than GPR, but from the drying method point of view, significantly ($p<0.05$) higher values were obtained for SGPR_L. These results can be probably due to the fact that lyophilization did not deteriorate the cellular structure during dehydration and the phenolic compounds such as anthocyanins and the flavonols which are often found in the external areas of the vacuoles are retained in the material. GPW_O presented a TPC significantly ($p<0.05$) higher compared to that of GPW_L, but there was no significant ($p>0.05$) difference in AA among these samples. In the case of SGPW_O, the TPC was 1.2 times higher compared to that of SGPW_L samples, whereas no significant ($p>0.05$) difference in AA was found among the samples. Indifferently of the drying methods applied, SGPW presented a higher TPC that GPW. Taking into account these results and the energy consumption in lyophilization process, oven-drying could be a good option for GP preservation for its future use in extrudate snacks.

Keywords:

Grape pomace; Lyophilization; Oven-drying; Polyphenols

Acknowledgments:

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P 139 BIOACTIVITY OF LACTOBACILLUS-FERMENTED PLANT BEVERAGES ON STRESSED COLON CELL AND *C. elegans* MODELS

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Fermented foods have been historically a part of our diets. In addition to preserving food and to making certain foods edible, fermentation also generates transformed and new bioactives compounds posing advantageous functional properties. Existing observational studies link regular consumption of fermented foods with a wide range of health-promoting attributes, raising public interest towards these products containing probiotics. According to the consumers' demand and given the trend to evolve towards more sustainability, fermented plant beverages represent one of the best opportunities. However, further scientific evidence of a beneficial health value of such functional fermented plant beverages is still needed. Thus, in the present study, Lactobacillus-fermented plant beverages have been produced from thyme, rosemary, pomegranate and tea using two Lactobacillus strains, one isolated from fermented black carrot juice and a *Lactobacillus plantarum* strain. Some bioactives compounds provided by these new food products, mainly (poly)phenol metabolites, have been identified by LC-MS spectrometry analysis. Fermented products have been then pasteurised before assessing their bioactivity on stressed colon cells and using *Caenorhabditis elegans* models. Anti-inflammatory potential of the fermented products has been assessed measuring the cytokine(s) released from CACO-2 cells and the expression of some key genes. Longevity, survival, oxidative stress and lipid accumulation have been investigated in *C. elegans* fed with Lactobacillus-fermented products. This study aims to provide scientific evidence that may be valuable for devising future strategies such as choosing promising fermented plant beverages and their derived bioactive compounds to make functional foods due to their potential relevance to human health benefits.

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P 140 COMMERCIALLY AVAILABLE AUSTRALIAN INDIGENOUS PLANTS AS A BASIS FOR DEVELOPMENT OF FUNCTIONAL FOOD PRODUCTS

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Background:

Australia has many Indigenous native plants that were used as a food source and as a medicine (*Bush Tucker*) for several millennia. The records about their preparation, consumption and health effects are predominately acknowledged in books. Their potential beneficial health effects were ascribed to their high phytochemical and predominately polyphenolic content. Polyphenols are secondary plant metabolites and their consumption has been associated with number of beneficial health effects. Previous studies of the Australian Indigenous plants have reported very high total polyphenolic content of these fruits (Figure 1). These native plants are also becoming popular on the Australian market however, their use is mainly as a part of the "Westernised" food products (crisps, jams). Nevertheless, a relative handful of Australian native plants have already been highlighted for the basis of new food products however research has not expanded beyond a *niche* group of plants. The characteristics of Australian native fruits have the significant potential to be used as a bioactive functional ingredient in developing functional food products that can be potentially used for management of several chronic conditions. Therefore, the aim of this study is to determine the total polyphenolic and flavonoid content of some of the commercially available dried Australian Indigenous plant foods.

Figure 1: *Selection of Australian Indigenous plant foods (Image by Tourism NT).*

Methods:

Selection (n=19) of commercially available dried Australian Indigenous plant samples (Davidson Plum (*Davidsonia jerseyana)*; Finger Lime (*Citrus australasica*); Muntries (*Kunzea pomifera*); Quandong Peach (*Santalum acuminatum*); Rosella (*Hibiscus sabdariffa*); Kakadu Plum (*Terminalia ferdinandiana)*; Desert Lime (*Citrus glauca*); Cinnamon Myrtle (*Backhousia myrtifolia*); Strawberry Gum (*Eucalyptus olida*); Lemon Myrtle (*Backhousia citriodora*); Mountain Pepper Leaf (*Litsea cubeba*); Aniseed Myrtle (*Syzygium anisatum*); Wattleseed (*Acacia victoriae*); Peppermint Gum (*Eucalyptus radiata*); Old Mans Saltbush (*Atriplex nummularia*); Round Leaf Mint (*Prostanthera rotundifolia*); Lemongrass tea (*Maar tea*); Jilungin tea (*Terminalia canescens*); Gumby Gumby tea (*Pittosporum angustifolium*) and Wattleseed (*Acacia victoriae)* were purchased from a local supplier. Colour analysis was performed using the hand-held colourimeter (Konica, Minolta, US). The methanolic extractions (70%; 1:10 (w/v)) of bioactives was performed using a benchtop sonicator set at maximum power 30 min. Total polyphenolic content (TPC) was determined using the Folin-Ciocalteau method while total flavonoid content (TFC) was performed using the aluminium chloride method. Results (Mean±SE) are expressed as for TPC as µg/mL gallic acid equivalents (µg/mL$_{GAE}$) or for TFC as µg/mL Catechin equivalents (µg/mL$_{CE}$)

Results:

There was a large variation in polyphenolic and flavonoid content in all samples. The TPC levels varied between 118.18 ± 11.61 (µg/mL$_{GAE}$) in Lemongrass tea, to 11114.89 ± 1806.06 (µg/mL$_{GAE}$) in Jilungin tea. The levels of TFC ranged between 116.53 ± 69.62 (µg/mL$_{CE}$) in Old Mans Saltbush to 3153.56 ± 651.05 (µg/mL$_{CE}$) in Mountain Pepper Leaf.

Conclusion:

The findings of this study further support the previous literature in Australian Indigenous plants and provide a future opportunity towards the development of functional food products that may be used in the management of several different chronic conditions. It is also important to establish new product development in partnership with local Indigenous communities.

P 141 MODULATION OF MULTIDRUG RESISTANCE IN BREAST CANCER CELLS BY CANNABINOIDS AND TERPENOIDS: CYTOTOXICITY AND THERAPEUTIC POTENTIAL

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Breast cancer is a prominent and critical disease in modern medicine, representing the most frequently diagnosed cancer in women worldwide. Current therapeutic approaches for breast cancer primarily involve surgery and chemotherapy. However, the development of multidrug resistance (MDR), caused by the overexpression of the transmembrane pump P-glycoprotein (P-gp), complicates the treatment of this disease. Substances found in Cannabis sativa L., such as cannabinoids and terpenoids, possess significant potential for anticancer therapy and modulation of MDR.

In this study, we examined the cytotoxicity and ability to modulate MDR of representative cannabinoids and terpenoids using human renal proximal tubular epithelial cells (RPTEC), breast cancer cells (MCF-7), and its paclitaxel-resistant sublineage (MCF-7/PAX). We evaluated collateral sensitivity (CS) and selectivity index (SI) in both tumor and non-tumor cell lines. The modulatory activity of P-gp was assessed by measuring the fold change in paclitaxel concentration and the accumulation of the fluorescent substrate Rhodamine 123. Our findings revealed that both cannabinoids and terpenoids exhibited mild antitumor effects compared to the non-tumor cell line RPTEC. Although terpenoids generally displayed low CS, β-Caryophyllene demonstrated up to six times higher toxicity against the resistant MCF-7/PAX than the non-resistant MCF-7 cell line. The terpenoid α-pinene predominantly modulated resistance in the tumor cells at a concentration of 100 µM. Among the cannabinoids, cannabidivarin (CBDV) and cannabinol (CBN) exhibited the most pronounced effect on resistance suppression at a concentration of 10 µM. However, no significant impact of CBDV and CBN on P-gp activity was observed. Based on our study, we suggest that cannabinoids may affect the gene expression of the P-gp efflux pump rather than directly inhibit its function. Future investigations will focus on exploring the modulation of P-gp expression by the aforementioned substances to gain a deeper understanding of their mechanism of action.

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P 142 POLAR LIPIDS FROM AVOCADO WASTES: DISCOVERING THE BIOACTIVE PROPERTIES

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Avocado consumption has standing out worldwide due to the recognition of its nutritional value and health benefits. However, either avocado consumption or its processing industry produces large amounts of wastes, as seeds and peels that are commonly discarded, although they are potential sources of healthy lipids, namely essential polyunsaturated fatty acids (PUFAs) like C18:3*ω*-3 and C18:2*ω*-6. These PUFAs are mainly esterified to polar lipids, such as phospholipids and glycolipids, which have already been identified in other matrices as added-value bioactive compounds with antioxidant and anti-inflammatory properties. Nonetheless, both the polar lipid signature of avocado wastes and its bioactive properties remain poorly explored. This work aims to *(a)* bioprospecting the total lipid extracts of avocado peel and seed as a source of bioactive compounds with anti-inflammatory and antioxidant properties and *(b)* correlate the bioactive potential with the lipidome signature of peel and seed characterized by C18-HPLC-ESI-HR-MS/MS to pinpointed structure activity relationship. The *in chemico* antioxidant activity of the lipid extracts was assayed by DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and ABTS$^{\bullet+}$ (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assays. The anti-inflammatory potential was also evaluated *in chemico* using the cyclooxygenase-2 (COX-2) inhibition assay. All the extracts demonstrate antioxidant activity, being able to scavenge both ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ (up to ≃40% DPPH$^{\bullet}$ inhibition and up to ≃80% ABTS$^{\bullet+}$ inhibition), depending on the concentration of the lipid extracts. Peel extracts presented the greater anti-inflammatory potential (≃20 to 90% COX-2 inhibition). MS/MS data showed that avocado peel and seed extracts presented a distinct and characteristic lipid signature both rich in polar lipids with *ω-3* and *ω-6* PUFAs. Overall, the results showed that the lipid extracts from avocado by-products can be a natural source of compounds with bioactive potential. This knowledge is important to valorize these wastes maximizing the retrieved add-value compounds and contributing to the sustainable exploitation of avocado.

Acknowledgments:

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P 143 CENTRIFUGAL PARTITION CHROMATOGRAPHY FOR DOWNSTREAM ISOLATION OF BLUISH ANTHOCYANIN-DERIVED PIGMENTS OBTAINED FROM BLUEBERRY SURPLUS

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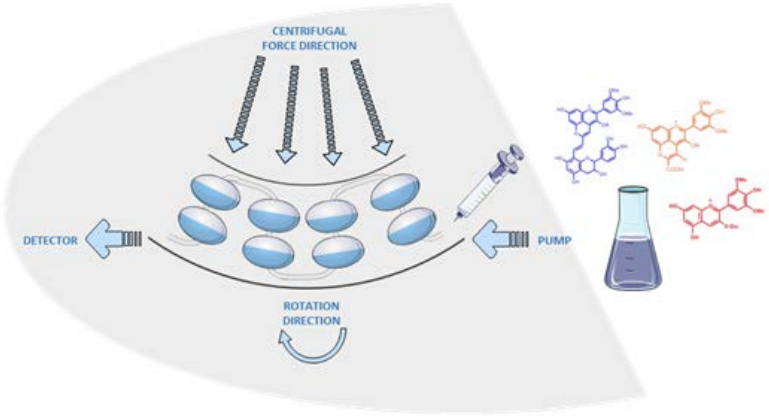
The demand for natural blue colorants has increased and with this the search for new molecules that can produce different color hues for application in food and nutraceutical industries. Portisins are bluish anthocyanin-derived pigments that were found to occur in red Port wines. These pigments are conventionally purified using reversed phase column chromatography (RP-CC). However, there are limitations to the implementation of column chromatography processes at industrial scale. In this work, centrifugal partition chromatography (CPC) process was developed as a more efficient and sustainable alternative to RP-CC for the preparative-scale purification of portisins. The strategy began with the extraction of anthocyanins from blueberry surplus and hemi-synthesis of respective portisins. Then, the CPC method development started with the biphasic solvent system selection followed by the chemometric based multivariate optimization of the CPC process parameters and ended up with a comparison with RP-CC. Aiming at maximizing the portisin content, process throughput, process efficiency, and minimizing the environmental risk factor, the effect of sample load (2–10 mg/100 mL), mobile phase flow rate (10–20 mL/min), and rotation speed (1000–1600 rpm) was assessed. The best biphasic solvent system and operating conditions were selected, allowing the purification of portisins by 5-fold. Overall, the proposed strategy demonstrated to be an effective and suitable alternative for the production and isolation of bluish portisin pigments with potential application in the nutraceutical and food industries, contributing to the sustainable utilization of these blueberry residues.



P 144 PHYTOCHEMICAL ANALYSIS AND TOXICITY OF MALAYSIAN *Hibiscus sabdariffa* L. (ROSELLE) CALYCES

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Abstract: Background: *Hibiscus sabdariffa* (roselle) calyces have gained popularity as natural and bioactive phytochemicals, known for their potential anti-hypertensive, hepatoprotective, antihyperlipidemic, and anti-obesity properties [1]. In this study, we evaluated the phytochemical composition of roselle calyces and conducted acute and sub-acute toxicity assessments of the water extract (Si-Roja). The findings serve as a crucial foundation for further research on developing this plant as herbal medicine.

Methods:

The phytochemical analysis of roselle calyces was performed using HPTLC and UPLC. Acute toxicity tests were conducted on six female rats using a single oral dose of Si-Roja at 300 mg/kg and 2000 mg/kg. For sub-acute toxicity assessment, 60 rats (30 female and 30 male) were divided into six groups of ten rats each. Si-Roja was administered daily at doses of 2000, 1000, and 500 mg/kg body weight for 28 days. Hematological and biochemical parameters were evaluated, along with histopathological analysis of the kidney and liver.

Results:

The phytochemical analysis revealed the presence of anthocyanins, including cyanidin-3-*O*-sambubioside, delphinidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside, and delphinidin-3-*O*-glucoside in the roselle calyces. The acute toxicity study showed no signs of toxicity, mortality, or morbidity at the dose levels tested. In the sub-acute study, Si-Roja did not significantly affect parameters such as white blood cell count, mean corpuscular volume, mean corpuscular hemoglobin concentration, lymphocyte count, platelet count, total bilirubin, or direct bilirubin. However, there were significant increases in aspartate aminotransferase (AST) levels in the medium and high dosage groups of female rats, as well as significant increases in alkaline phosphatase (ALP) and alanine aminotransferase (ALT) levels in male rats.

Conclusion:

The results of this study indicate that Si-Roja did not exhibit acute or sub-acute toxicity in Sprague Dawley rats following oral administration for 14 and 28 days. However, caution is advised due to the observed significant increase in AST, ALP, and ALT levels in certain groups. Further research is recommended to explore the potential implications of these findings.

Keywords:

Roselle; Anthocyanins; Toxicity

Reference:

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P 145 THE SYNTHESIS OF UNSATURATED ESTERS VIA ENZYMATIC PROMISCUOUS KNOEVENAGEL CASCADE

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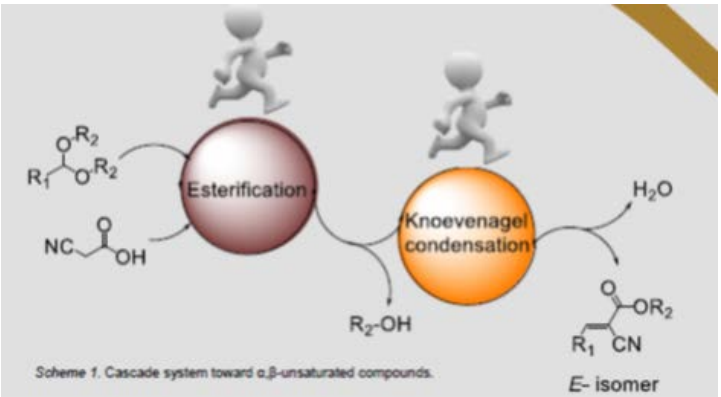
Knoevenagel condensation continues to be of outstanding importance in the way of building complex molecules, being commonly used in the chemical, pharmaceutical, and food industry [1].



Scheme 1. Cascade system toward α,β-unsaturated compounds.

Classically, the reaction between the carbonyl and active methylene compounds occurs in the presence of an organic base or Lewis acid. Herein, a new enzymatic protocol based on a lipase-catalysed cascade toward (E)- α, β- unsaturated carboxylic acid esters will be presented. The proposed cascade consists of elementary organic processes starting from acetal and cyanoacetic acid leading to the formation of desired products in a cascade sequence. The combination of promiscuous abilities gives a new opportunity to synthesize complex molecules in the one-pot procedure.

We disclose a new route for the synthesis of desired products via tandem process based on the enzymatic esterification of cyanoacetic acid and a subsequent Knoevenagel reaction. (Scheme 1). This strategy involves using acetals as a precursor to aldehyde and simultaneously an alkoxy group donor for the enzymatic esterification of cyanoacetic acid followed by a Knoevenagle reaction leading to α,β- unsaturated compounds.

We have noticed that the reaction medium is the key factor affecting the reaction efficiency and showed a dramatic difference in the formation of desired products. Conducted experiments clearly revealed a correlation between log P of the solvent and reaction efficiency.

Also multiple applications of enzymes did not diminish activity.

The results of our studies will be presented and the influence of enzyme, solvent, and reaction conditions on the cascade process will be discussed [2].

Acknowledgment:

We gratefully acknowledge the financial support of the National Science Centre Poland for the OPUS 2019/33/B/ST4/01118.

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P 146 EFFECT OF HIGH-POLYPHENOL CONTENT COCOA ON COGNITIVE AND EMOTIONAL BEHAVIOR IN MICE

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Background:

Cocoa consumption has recognized health benefits. Cocoa contains more than 300 different constituents, including minerals, fibre, methylxanthines and polyphenols, the latter being the main components. The health benefits of cocoa have been long recognised and can be attributed to the potent activity of cocoa polyphenols, mainly flavanols, which are found in cocoa in higher concentration than in other plant-derived foods-i.e., 0.46–0.61 g/kg flavanol monomers and 4–5 g/kg flavanol polymers-. These compounds benefit the cardiovascular system, have anti-inflammatory properties, reduce insulin resistance, and enhance the growth of beneficial gut bacteria. In addition, cocoa consumption has numerous health effect related to the enhancement of cognitive function, although further research is needed regarding the actions of cocoa on emotional and cognitive behavior.

Objectives:

This study aims at selecting a high-polyphenol content cocoa (HPC) and a low-polyphenol content cocoa (LPC) for further evaluation of the effect of phenolic compounds, especially, flavan-3-ols on anxiety-like behavior, exploration, and hippocampal-dependent learning.

Methods:

Different cocoa powders were evaluated in terms of total phenolic content (TPC) through the Folin-Ciocalteu assay, procyanidin contents using HPLC-FLD-DAD, and antioxidant activity, which was assessed through DPPH, ABTS and FRAP assays. Then, two cocoas were selected. One with the highest TPC, procyanidins and antioxidant activity (HPC) and other with the lowest values (LPC). Young adult male and female C57BL/6J mice were fed standard diet (CTR), HPC diet or LPC diet for 4 weeks. The mice were evaluated in a battery of behavioural tests (elevated plus maze test, open field test and novel object and place recognition tests).

Results:

The selected HPC had a TPC of 57.4 mg of gallic acid equivalent (GAE)/g d.w., 28.58 mg of catechin equivalents (CE)/g d.w. and antioxidant activity of 97.94, 267.43 and 98.74 mg Trolox Equivalents (TE)/g d.w. for DPPH, ABTS and FRAP, respectively while the LPC had a TPC of 9.2 mg GAE/g d.w., 3.30 mg CE/g d.w., and 30.77, 73.97 and 28.88 mg TE/g d.w. Both diets increased food consumption across the first four weeks compared to CTR; however, cocoa consumption did not have a clear effect on body weight gain. Cocoa-enriched diets did not influence general exploratory activity and anxiety-like behaviour in the elevated plus maze test; however, a decreased locomotor activity was found in mice fed with LPC diet in the open field test. HPC mice showed increased object recognition memory, while all groups performed similarly in the place memory test. When sex differences were investigated, sex did not modulate the effects.

Conclusion:

Cocoa-enriched diets, specifically the HPC diet, could enhance object memory. In this regard,polyphenols (flavan-3-ols particularly) seem to be responsible for the different cognitive effects observed between the HPC and LPC diets. In addition, cocoa-enriched diets appear to be sensorially accepted, as they increase appetite for food consumption.

P 147 APPLICATION OF ULTRAFILTRATION TO PRODUCE SHEEP'S AND GOAT'S WHEY-BASED SYNBIOTIC KEFIR PRODUCTS

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Membrane filtration technologies are the best available tools to manage dairy byproducts such as cheese whey, allowing for the selective concentration of its specific components, namely proteins. The aim of this work is the development of new synbiotic kefir products based on sheep and goat liquid whey concentrates (LWC) obtained by ultrafiltration. For each LWC (sheep's or goat's) four formulations based on a commercial kefir starter or traditional kefir were produced: commercial kefir (EK); commercial kefir with probiotic culture (EKABT5); traditional kefir (TK); traditional kefir with probiotic culture (TKABT5). The physicochemical, microbiological, and sensory properties of the samples were determined. Membrane process parameters indicated that ultrafiltration can be applied for obtaining LWCs in small/medium scale dairy plants with high protein concentration (16.4% for sheep and 7.8% for goats). Sheep kefirs showed a solid-like texture while goat kefirs were liquid. Although further work must be undertaken to improve the acceptability of the products It could be concluded that small/medium-scale dairy plants can use ultrafiltration equipment to valorize sheep's and goat's cheese whey-producing synbiotic kefirs. Sheep kefir products presented a solid-like texture while goat kefir products were liquid. Regarding sheep kefir products, the highest values of G' were obtained for TK and TKABT5. EK and EKABT5 presented G' values *ca.* one log cycle lower as compared to the ones produced with the traditional kefir. These observations were confirmed by the evaluation of the complex viscosity. In all the goat kefir products, the viscous modulus (G") was higher than the elastic modulus (G'), indicating the liquid nature of the products, which is also confirmed by their complex viscosity. All samples presented counts of lactic acid bacteria higher than log 7 CFU/mL, indicating the good adaptation of microorganisms to the concentrated whey matrixes.

Keywords:

Cheese whey; Ultrafiltration; Ovine; Caprine; Kefir; Prebiotics; Probiotics

Funding:

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P 148 THE EFFECT OF 4-METHYLCATECHOL ON VASCULAR SMOOTH MUSCLE CELLS *EX VIVO* AND *IN VIVO*

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Background:

Natural polyphenols from the human diet, particularly flavonoids, exert cardioprotective effects despite their low bioavailability. This could be explained by the formation of active metabolites[1–3].

Objectives:

The aim of this work was to verify the vasoactivity of the colonic metabolite 4-methylcatechol (4-MC) in both *ex vivo* and *in vitro* models and to study the mechanism(s) of this action.

Methods:

Ex vivo isolated rat aorta and mesenteric artery models with isometric tension measurements were used as well as *in vivo* spontaneously hypertensive rats with blood pressure and heart rate recording. The mechanism of vasodilatory effects was investigated *ex vivo* using specific activators/inhibitors (ODQ, DT-3, RP-8-pCPT-cGMPs, forskolin, nitroprusside, BayK8644, iberiotoxin, 4-aminopyridine, linopirdine, glybenclamide, Ba^{2+}).

Results:

4-MC produced vasodilatory effects *ex vivo*, which were more pronounced on the small mesenteric artery. This corresponded to observed blood-pressure-lowering effects *in vivo* after both i.v. bolus administration and i.v. infusion. No effects on heart rate were observed. In mechanistic experiments, the vasodilatory effects of 4-MC were dependent on the activity of K_v channels expressed in the vascular smooth muscle. An important, but not exclusive, role played the K_v7 subtype which is abundant in peripheral vessels.

Conclusion:

The colonic metabolite of polyphenols, 4-MC, exhibits *ex vivo* vasodilatory and *in vivo* antihypertensive effects, which may be explained by direct action on vascular smooth muscle and associated with potassium channel activity.

Acknowledgement:

Project of the Czech Research Health Council (NU21-02-00135).

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P 149 UP4HEALTH INGREDIENTS: SPECIALITY PLANT-BASED FOOD PROCESSING BY-PRODUCT EXTRACTS FOR INDUSTRIAL APPLICATION

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The UP4HEALTH project aims to recover biomolecules with high added value from food processing by-products (grape pomace, olive pomace and olive pits and nut by-products) on a pre-industrial scale in an integrated biorefinery. The intention is to convert these by-products into healthy and sustainable ingredients for the nutraceuticals and cosmetic sectors. It is important to highlight that the UP4HEALTH ingredients will meet the requirements of the consumer and industry markets in these sectors.

So far, the partners are testing polyphenol-rich extracts and prebiotic xylooligosaccharides obtained from by-products of the olive oil industry. The ongoing assays include, therefore, the extracts obtained directly from the olive pulp (PBF^{+}) and olive pulp extracted by supercritical fluid extraction with CO_2 (PBF). A third promising extract (XOS) was obtained from olive crushed pits. It consisted of a soluble prebiotic fibre extract designed to promote intestinal regularity and digestive health, promoting the growth of probiotic organisms. These oligomers have low calories, permitting their utilisation in anti-obesity diets.

After the chemical characterisation of the extracts, their bioactivities were tested in order to develop functional products incorporated with such extracts. Thus, the antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties of the UP4HEALTH ingredients were evaluated. To assess the potential of the ingredients for incorporation into cosmetics, *in vitro* tests of anti-tyrosinase activity are also being carried out.

The best results regarding the antioxidant activity evaluated through the thiobarbituric acid reactive substances (TBARS) assay in brain cell homogenates and the cellular antioxidant activity (CAA) assay were obtained for the polyphenol-rich extracts (PBF and PBF+). Moreover, the three ingredients inhibited some pathogenic bacteria, especially Gram-positive. Regarding the cytotoxic activity evaluated in three human tumour cell lines, the UP4HEALTH ingredients inhibited the growth mainly of AGS (human gastric adenocarcinoma) and Caco2 (colorectal adenocarcinoma) cells. Moreover, when tested in primary cell cultures from the porcine liver (PLP2), the samples did not show cytotoxicity, proving their safety for use. Regarding the anti-inflammatory activity, evaluated using a mouse macrophage-like cell line (RAW 264.7), the best results were obtained with the XOS extract. Concerning the anti-tyrosinase activity, the tests are still being developed. However, for some samples, low bioactivity has already been verified.

The following steps will include bioavailability studies of the extracts and some of the final products already being developed so that it is possible to validate the UP4HEALTH ingredients in food supplements and cosmetics for end users.

Acknowledgements:

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P 150 FLAVONOIDS FROM HAWTHORN AND THEIR *EX VIVO* VASODILATORY EFFECTS IN PORCINE CORONARY ARTERIES ARE MEDIATED BY L-TYPE CALCIUM CHANNELS

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[1] *Faculty of Pharmacy, Charles University, Pharmacology and Toxicology, Hradec Králové, Czech Republic*

Background:

Hawthorn administration was documented to produce beneficial effects in cardiovascular diseases which may include vascular effects[1–3]. So far, the responsible compound(s) and the mechanism of action are unknown.

Objectives:

The aim of this work was to investigate the possible vasoactivity of principal hawthorn constituents and to study the mechanism(s) of this action.

Methods:

Isolated porcine coronary arteries precontracted by KCl were used for screening of vasoactive properties of the principal constituents of the hawthorn extract by *ex vivo* isolated tissue bath system and these effects were compared to nitroprusside sodium. In a separate set of experiments, the mechanisms of vasodilatory action of the two most active substances, tamarixetin and isorhamnetin, were determined using various activators/inhibitors and endothelium intact or denuded vessel rings.

Results:

Various constituents of the hawthorn extract showed different *ex vivo* vasodilatory properties with isorhamnetin and tamarixetin being the most potent (EC_{50}=47 and 48 µM, respectively). In mechanistic studies, the vasodilatory action of both substances was shown to be mediated by L-type of Ca^{2+} channels expressed in the vascular smooth muscles, and the effect was dose-dependent. In contrast, no significant effects on vascular guanylyl cyclase, PKG or PKA pathways, and various K^+ channels (BK_{Ca}, K_V, K_{IR}, K_{ATP}) were found.

Conclusion:

Isorhamnetin and tamarixetin, the most active components of hawthorn extract, exhibit dose-dependent vasodilatory effects in porcine coronary arteries, which are mediated by inhibition of L-type of Ca^{2+} channels on vascular smooth muscles.

Acknowledgment:

This work was supported by the grant agency of Charles University (No. 236923).

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Keywords:

Isorhamnetin; tamarixetin; Porcine coronary artery; *Ex vivo*, Vasodilatory; L-type calcium channel

P 151 MODULATION OF EXPRESSION OF COLON CANCER BIOMARKERS BY DAILY BERRY CONSUMPTION

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Background:

Signalling pathways that play a role in colorectal cancer development can be modulated by diet. Berries are suggested to have anticancer properties due to their high content of fibre, vitamins, and bioactive compounds such as polyphenols. On the other hand, high consumption of red and processed meat, typical in Western diet, is known to facilitate the formation of endogenous N-nitroso compounds, which are known carcinogens.

Objective:

The aim of this research was to evaluate whether cancer biomarkers can be affected by supplementing a habitual diet rich in red and processed meat with a mixture of berries with high polyphenol content in a four-week clinical trial with healthy adult volunteers.

Methods:

A clinical trial with 43 healthy volunteers divided in two diet groups was carried out. Control diet contained 150 g of pork meat/day and experimental group of 150 g of meat and 200 g of berries per day. Stool samples were collected from the study participants at the beginning and at the end of the 4-wk trial. The concentration of N-nitroso compounds in the faeces was analysed. Caco-2 and HCA-7 colon adenocarcinoma cells and HaCaT cells, representing non-cancerous epithelial tissue, were exposed in 2D and 3D cultures to faecal water extracted from stool samples. Genotoxicity marker and expression of markers for the Wnt/β-catenin and PI3K/AKT/mTOR pathways, the cell proliferation marker Ki67, and the apoptosis marker cleaved caspase-3 were detected by Western blotting and immunofluorescence staining methods.

Results:

We found that adding berries to a diet with relatively high red meat content led to a modulation of the Wnt/β-catenin and PI3K/Akt/mTOR pathways. The expressions of βcatenin, PS6 and Ki67 were downregulated in colon cancer cells exposed to faecal water of volunteers who had followed a diet supplemented with berries, causing a subsequent decrease in cell proliferation. Even though concentrations of carcinogenic N-nitroso compounds were not affected by the diet, consumption of 200 g/day of berries seemed to reduce the DNA double-strand break formation in HCA-7 cells exposed to the faecal water from the Meat&Berries group.

Conclusion:

Our results suggest that consuming berries as part of a habitual Western-type diet could lead to less cancerous colon metabolism and possibly lower the risk for colorectal cancer by modulating the central signalling pathways in cancer.

P 152 ANTI-INFLAMMATORY ACTIVITY OF COMMERCIALLY AVAILABLE DIETARY SUPPLEMENTS

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Dietary supplements can support a healthy state of the organism, provide the necessary amount of nutrients and prevent several diseases. The anti-inflammatory activity of supplements can reduce inflammation, especially chronic inflammation that is associated with heart diseases, diabetes, or autoimmune diseases. Furthermore, anti-inflammatory activity can be manifested by supporting the immune system or protecting against oxidative stress.

In this work, the anti-inflammatory effects of three commercially available capsules sold as a dietary supplement were investigated. The anti-inflammatory effect was observed as a decrease in the level of released nitric oxide (NO) and the cytokines interleukin 6 (IL-6) and tumour necrosis factor alpha (TNFα) on the mouse macrophage cell line (RAW 264.7). All three capsules had an effect on NO release. Capsules containing a high content of L-glutathione, quercetin, resveratrol, epigallocatechin gallate, selenium and zinc had the strongest effect. This capsule was also the only one capable of reducing the release of cytokines. Based on the results, this capsule could have an effect on the prevention of inflammatory diseases.

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P 153 SOUR CHERRY (*Prunus cerasus*) JUICE DECREASES STARCH BIOACCESSIBILITY OF WHITE BREAD

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Consumption of certain fruits, may have an effect on starch digestion. The proposed effect is often associated with polyphenols as well as the acidity of the given food. Sour cherry *(Prunus cerasus*) juice from Hungarian cultivar (Érdi bőtermő) was tested during *in vitro* co-digestion with white bread in order to gather information on its effect on starch digestion. Digestion simulation of white bread was performed after *in vivo* oral phase mastication followed by semi-dynamic *in vitro* gastric phase and static small intestinal phase. Sour cherry fruit was obtained from Hungarian farmers and juice was prepared in the laboratory by simple pressing (no enzyme treatment) and Pasteurisation before bottling. Co-digestion experiments in 1:2 weight ratio of in-house baked standard white bread and i) sour cherry juice or ii) a sour cherry juice control solution containing 5.6% glucose, 4.5% fructose, 2.4% galactose and 1.3% malic acid (based on the composition of the cherry juice) were performed. Samples taken at several time points (15, 30, 45, 60, 90, 120, 150, 180, 210, 240 min) during the digestion simulation, were centrifuged (10000 rpm, 10 min, 4°C), and supernatants were analysed to quantify the released starch after selective polysaccharide precipitation in an 80% (v/v) ethanol solution (oligosaccharides with degrees of polymerization up to 10). Released starch was quantified as glucose equivalent after complete hydrolysis by amyloglucosidase.

Results showed that the addition of sour cherry lowered starch digestion of white bread. The *in vitro* assessed glycaemic load – based on AUC calculation – was decreased by 34% (in absolute terms) with sour cherry and by 25% with the control solution. In addition, the co-digestion with the control solution highlighted that the effect is not only due to the inherently acidic nature of sour cherry – which could rapidly inactivate action of alfa-amylase during gastric phase- but also due to the additional components present in sour cherry. These results highlight the effect of acidic environment on carbohydrate digestion moreover its importance over additional compounds such as polyphenols usually appointed to inhibition of amylolytic enzymes.

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P 154 FT-IR FINGERPRINT AND BIOACTIVITIES OF CERATONIA SILIQUA FRUIT OF CRETAN ORIGIN

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The evergreen carob tree, *Ceratonia siliqua L.*, is found in the dry and semi-arid areas of the Mediterranean basin. Carob fruit is used to create a variety of foods and drinks. All genetic, cultivar, seasonal, and environmental influences affect its composition. We aimed at providing the spectral signature and bioactivities of a phenotypically distinct variant located in Pines, Crete (coordinates: L:35,278545, A:25,715529). The powder, obtained by grinding the carob fruit, was analysed applying FT-IR, utilising a diamond ATR compartment (ATR-FTIR), adopted for a fast and non-destructive screening. The spectrum of the variant was compared to these of three commercial samples. Characteristic peak-indexes, or the absence/presence of specific peaks were marked for each sample, i.e. mostly molecular vibrations related to aliphatic compounds, carbohydrates and proteins were reported. The target variant was characterised by the absence of peaks at 1692 (C=O stretching vibration, amide I band), 1325 (aliphatic C-H stretching vibration/ CH_2 wagging) and 1209 cm^{-1} (CS/PO/SO stretching vibrations) which were present in one or more of the spectra of the commercial samples. In parallel, the fruit of the target variant was subjected to cold extraction in methanol/water and compounds such as sucrose, glucose, fructose, pinitol and myo-inositol were identified and quantified applying ESI-MS. In colon adenocarcinoma cells, the extract was found to possess cytostatic activity applying the MTT assay, while being marginally inhibitory for normal cells. Based on the DCFH-DA cellular internalization and oxidation assay and on the DPPH assay the extract was found to exhibit significant antioxidant activity.

P 155 A STANDARDIZED NUTRACEUTICAL SUPPLEMENT IMPROVES PAIN, QUALITY OF LIFE AND INFLAMMATION IN KNEE OSTEOARTHRITIS PATIENTS; A RANDOMIZED CLINICAL TRIAL

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Osteoarthritis (OA) is the most common form of arthritis with increasing prevalence. With symptoms such as pain and stiffness that spur out from chronic inflammation and oxidative stress (OS), the quality of life (QoL) of the afflicted is seriously hindered. As it has no cure, multipotent bioactive constituents of the foods are emerging as promising therapeutic alternatives. Such metabolites are polyphenols with established positive effects on human's health. On that note, a randomized blinded, parallel-group clinical trial was conducted in 60 patients with knee OA in order to evaluate a standardized polyphenolic supplement in the management of pain, inflammation, OS and in the health-related QoL of the patients. Subjects with moderate/severe symptoms were randomized to receive either a polyphenol supplement (curcuma phospholipid (148.4 mg), rosemary extract 40% (51.9 mg), resveratrol 98% (51.9 mg), ascorbic acid (29.7 mg)), or an active comparator (ascorbic acid 29.7 mg) twice, daily for 12 weeks. Primary outcomes were changes in visual analogue scale (VAS) score and in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale. Secondary outcomes were changes in WOMAC stiffness and functionality subscales, in ShortForm36 (SF-36) and in levels of inflammatory and OS biomarkers. Data were analyzed in an intention to treat basis. Post intervention VAS, WOMAC pain, physical function and total score decreased significantly ($p<0.001$ for all) in the group that received the polyphenols and the mean changes between the two groups were statistically significant ($p=0.011$, $p=0.013$, $p=0.047$, $p=0.048$ respectively). Also, the mean changes between the two groups were statistically significant for SF-36 bodily pain and physical functioning ($p=0.013$, $p=0.023$ respectively) and for tumor necrosis factor-alpha (pg/ml), C-reactive protein (mg/L) and myeloperoxidase (ng/ml) ($p=0.034$, $p=0.016$, $p=0.010$, respectively). The standardized supplement alleviated symptoms and improved inflammatory/OS biomarkers in KOA patients supporting the case of the use of combined polyphenols in OA.

P 156 A PIG MODEL FOR INTESTINAL PERMEABILITY

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The interaction between nutrition, gut health and chronic diseases is receiving increasing attention. Intestinal barrier defects are implied in several chronic, non-communicable diseases and representative models are needed to further research in this field. Due to their omnivorous diet and similar anatomy and physiology, pigs are excellent nonclinical model animals to investigate intestinal health. By comparing two methods to disturb intestinal homeostasis, we developed a relevant model for intestinal permeability.

Growing castrated male pigs (Hybrid sow x Piétrain boar, n=24), aged 13–14 weeks, were housed in metabolic cages and randomly assigned to different treatments during two separate experiments: dextran sodium sulfate (DSS, 7 days, n=8) treatment was compared with control (7 days, n=4) or indomethacin (3 days, n=8) treatment was compared with control (3 days, n=4). After an overnight fast, baseline urine was collected and a solution of sucralose and erythritol was administered to assess intestinal permeability. Urine was collected per pig and recovery of sugars in urine was quantified using UHPLC-HRMS. This study was approved by the local ethical committee (EC 2022-410).

DSS treatment did not affect permeability, whereas indomethacin altered intestinal permeability, as indicated by increases in sucralose excretion and sucralose:erythritol ratio in urine of treated vs control pigs. These results show opportunities to study intestinal permeability in pigs as a relevant animal model for human health. In the future, this model could be used to test protective effects of diet or isolated dietary compounds.

P 157 FUNCTIONAL STUDIES OF VEGETABLE AND FRUIT MOUSSES BEFORE AND AFTER ENRICHMENT WITH A FIBER PREPARATION OF POTATO STARCH

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Vegetable and fruit mousses are becoming more and more popular among consumers. These products contain soluble dietary fiber in the form of pectins, as well as insoluble fiber derived from undigested parts of cell walls, e.g. lignin, cellulose and hemicellulose. However, the total fiber content in one portion of the mousse isn't sufficient and is usually up to 2.3 g per 100 g of the product. New products with increased nutritional and health value are sought, which can be obtained by adding dietary fiber. As part of the "PreSTFibre4kids" project, Tymbark sp. z o.o. in cooperation with the Jan Długosz University in Częstochowa, produced vegetable and fruit mousses enriched with a fiber preparation made of potato starch. The aim of the study was to examine the functional properties of vegetable and fruit mousses before and after enrichment with a fiber preparation of potato starch. The research material consisted of 3 flavors of mousses: apple-peach-parsnip, apple-cherry-carrot and apple-carrot-quince. The scope of work included the measurement of pH and the analysis of the CIE L*a*b* color parameters of vegetable and fruit mousses, as well as the designation of the total dietary fiber content using the enzymatic-gravimetric method AOAC 991.43 in freeze-dried vegetable and fruit mousses. The addition of a fiber preparation made of potato starch caused a slight decrease in the pH of the apple-cherry-carrot mousse. A change in color to a darker one was observed for each flavor, with the apple-carrot-quince mousse having the greatest difference in color parameters (ΔE). Freeze-dried vegetable and fruit mousses enriched with a fiber preparation from potato starch were characterized by a higher total dietary fiber content than freeze-dried non-enriched mousses. On the basis of the functional studies carried out, it wasn't observed that the enrichment of vegetable and fruit mousses with a fiber potato starch preparation had a negative effect on the final product. It can therefore be concluded that the addition of a fiber potato starch preparation affects the functional properties of fruit and vegetable mousses, improving their nutritional and health value.

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P 158 DIFFERENTIAL EXPRESSION PROFILE OF BITTER TASTE RECEPTORS THROUGHOUT THE HUMAN GASTROINTESTINAL TRACT

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The main role of bitter taste receptors (TAS2Rs) in the oral cavity is to detect taste and identify toxins. TAS2Rs are also present in extraoral tissues, such as the brain, lungs or gastrointestinal tract (GIT), where they have been associated with various functions, although these have not yet been clearly defined. One of the most interesting tissues in which to analyze them is the GIT. In this tissue, the TAS2Rs are in direct contact with their possible ligands present in some foods. The aim of the work was to analyze which TAS2Rs predominate along the GIT in humans and the relevance of gender. We used samples of cheek mucosa, jejunum and colon from age-equivalent groups where we quantified the gene expression of several TAS2Rs by quantitative PCR. In the cheek mucosa of males, we observed that TAS2R3, TAS2R14 and TAS2R30 were the most highly expressed receptors, whereas TAS2R1, TAS2R38 and TAS2R42 were the least expressed. In the jejunum of females, TAS2R14 was the significantly most expressed receptor. On the other hand, TAS2R39 was the receptor with the lowest expression. In ascending colon, we saw that the most expressed receptors were TAS2R4, TAS2R14 and TAS2R31. Looking at the influence of gender, men showed higher levels, although the only significant differences were observed in TAS2R3, TAS2R14 and TAS2R42. In conclusion, we observed a different expression profile along the gastrointestinal tract, sensitive to gender modulation. Moreover, TAS2R14 is observed with a high level of expression in all tissues.

P 159 INTAKE OF PLANT BIOACTIVES IN THE HUMAN POPULATION

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Background:

Dietary habits are one of the main factors influencing the health status of an individual. It is well known that plant-based diets are associated with a reduction of several types of cancer, cardiometabolic diseases, and neurological disorders. Fruit and vegetables provide fibres, micronutrients and a wide range of biologically active compounds that can contribute to prevent chronic diseases. (Poly)phenols and carotenoids are the most studied phytochemicals, but many other plant bioactives can be involved in the promotion of the health status, like phytosterols, glucosinolates, alkaloids, thiosulfinates and alkylresorcinols, among others.

Objective:

This work aimed to describe the intake of all major dietary plant bioactives in the human population, considering (poly)phenols, terpenoids, *N*-containing compounds, and miscellaneous phytochemicals.

Methodology:

To evaluate the intake of these phytochemicals in the human population, literature searches were performed for the most important classes, subclasses, and individual compounds.

Results:

(Poly)phenols are the most consumed plant bioactive compounds and their intake can range between 664 and 1741 mg/day. In particular, flavonoids like flavan-3-ols and phenolic acids like hydroxycinnamic acids are the most representative (poly)phenols in the diet. Terpenoids are the widest compound family, including a huge variety of molecules like phytosterols (triterpenoids) and carotenoids (tetraterpenoids). The terpenoid highest intakes come from phytosterols, showing values around 250–400 mg/day for the general population and 600–800 mg/day for vegetarians/vegans. Carotenoids, instead, are consumed in smaller amounts (1 to 22 mg/day). One of the most representative *N*-containing compounds in the human diet is caffeine. Data show that European adults and the very elderly (>75 y) present the highest intake of this alkaloid (36–319 mg/day for adults and 22–417 mg/day for the very elderly, respectively). After caffeine, amines like tyramine, putrescine, and cadaverine, that can be found in pickled vegetables, beer and wine, are widely present in our diet. Miscellaneous phytochemicals gather many different compounds with variable intake data. For instance, alkylresorcinols are the most consumed and their intake can be higher in people who eat whole-grain cereals (36 ± 19 versus 44 ± 32 mg/day). Thiosulfinates, like alliin and its derivative allicin, show the second highest value of intake within this heterogenous family and their intake ranges from 7.8 to 17 and 3.6 to 7.8 mg/day, respectively.

Conclusions:

The amount of literature on the intake of plant bioactives in the human population is very different, depending on the compounds chosen. (Poly)phenols and carotenoids are the most studied phytochemicals, while there is a lack of information on many other plant bioactives. This makes establishing the intake of some compounds in the population difficult and, thus, it limits the knowledge about their role in human health. Further efforts to better assess the dietary intake of all plant bioactives are needed.

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P 160 APPLICATION OF MASS SPECTROMETRY IMAGING (MSI) FOR THE EVALUATION OF (POLY)PHENOL DISTRIBUTION *IN VIVO*: A REVIEW

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(Poly)phenols are organic compounds produced by plants as secondary metabolites and are abundantly present in fruits, vegetables, nuts, herbs, and other plant-derived products. Various studies have demonstrated the positive impacts of these compounds on human health. Nonetheless, the comprehension of the absorption and distribution mechanisms of phenolic compounds within the human body is of great importance for precisely identifying their bioactive form in target organs or tissues and consequently for understanding their biological effects.

Mass spectrometry imaging, or MSI, is a highly effective analytical method that provides a visual *in situ* representation of the molecular distribution within complex samples and biological tissues. This technique allows to create a map of various molecules in a tissue sample, providing spatial information that cannot be obtained through other analytical techniques.

The review aims to provide a useful and informative summary of the applications of MSI so far published for evaluating (poly)phenol distribution in animal tissues. The studies in which MS imaging was applied mainly dealt with *in vivo* interventions in mice and rats with any MSI technique, namely matrix-assisted laser desorption ionization (MALDI), desorption electrospray ionization (DESI), and secondary ion mass spectrometric (SIMS).

From this review, it emerges that the application of MSI for the study of phenolic compounds is yet to get a foothold but is expected to increase as researchers seek to advance their comprehension of the health-promoting attributes of (poly)phenols and their metabolites. MSI represents a viable approach for exploring the absorption and distribution of bioactive compounds in tissues, useful to enhance the comprehension of the pharmacokinetics of (poly)phenols and their metabolites, along with their possible site of action and transport mechanisms implicated in their distribution.

This work is part of the PNRR Partnership Extended ON FOODS consortium (ON Foods – Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods).

P 161 DEVELOPING AN *IN VITRO* MODEL OF THE INFANT GUT BARRIER VIA PERMEABILITY ENHANCERS

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In newborn infants, the intestinal barrier is permeable, allowing the absorption of large food molecules and bioactive components from breast milk. This enhanced permeability is part of a complex system that profoundly influences the growth and maturation of the infant's gut barrier. However, it can also lead to bacterial translocation and inflammation, resulting in necrotizing enterocolitis (NEC). Formula-fed infants are more likely than breastfed newborns to develop NEC. Next-generation infant formula should aim to promote gut maturity by reducing the permeability of the newborn's gut. To test infant formula for this health parameter, we need firstly to create an *in vitro* model of a leaky gut barrier to mimic the newborn.

Our lab has investigated several different compounds to create this *in vitro* model. Here we treated polarised Caco-2/HT29-MTX monolayers with different concentrations of the bile salt sodium glycodeoxycholate (GDC) for 2 hours. We examined the impact of the treatment on the monolayer›s integrity and overall health status. We also examined the ability of the monolayers to recover once sodium glycodeoxycholate was removed. Sodium glycodeoxycholate (0.8 mM) significantly reduced Trans-Epithelial Electrical Resistance values compared to media alone ($P<0.001$). MTS, LDH, and Caspase-3 assays indicate that this treatment does not affect the viability of the cells. Surprisingly, acidic mucins were significantly increased in the presence of 0.8 mM sodium glycodeoxycholate compared to media alone. Removal of the bile salt allowed recovery of TEER values within an 8 hour period.

Due to the increased permeability and the capacity for recovery, the sodium glycodeoxycholate-treated Caco-2/HT29-MTX monolayer shows promise as an *in vitro* model of a leaky gut barrier. Developing an *in vitro* gut barrier is an important first step to creating infant formulae that promote gut barrier maturation.

P 162 BIOAVAILABILITY AND EXCRETION PROFILES OF BETACYANIN PIGMENTS - VARIABILITY AND CORRELATION BETWEEN DIFFERENT ROUTES OF EXCRETION

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As abundant pigments in red beetroot, betacyanins are increasingly investigated for their bioactivities and bioavailability. The current work addresses the gap in knowledge regarding betalain bioavailability, transformation and excretion. Analysis of renal and fecal excretion profiles in humans after consumption of beetroot revealed very low bioavailability and fast elimination of pigments, while the majority of betalains underwent severe depletion during GI transit, evidenced by decarboxylation, deglucosidation and dehydrogenation. Betacyanin metabolite levels in human urine were positively associated with those in stools ($p<0.05$), indicating significant impact of pigment metabolism in the gut on their bioavailability. In addition, the current study revealed large inter-individual and compositional variabilities of pigment after colonic fermentation compared with systemic metabolism, likely attributed to the increasing complexity of intestinal environment with diverse gut microbiota. To conclude, intestinal uptake and systemic metabolism of betacyanins are intimately associated with their intestinal biotransformation, with gut microbiota serving as a crucial factor.

P 163 DEVELOPMENT OF AN ANIMAL MODEL FOR TESTING THE EFFECT OF DIET ON MEMORY

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The suitability of the chick as a model system for the study of molecular mechanisms involved in memory is well documented (Rose 2000). Cherkin (1969) developed a one trial passive avoidance learning task for one day old chicks based on their tendency to peck at small bright objects such as beads. The aim of this study was to develop an animal model based on passive avoidance (unpleasant stimulus) learning test for testing the memory in aged chicks (14 days old) allowing to be used for dietary interventions studies. A total of 40 one day old male broiler Cobb chicks obtained from a commercial hatchery were randomly distributed in in 10 pens (4 birds per pen), received a control diet for 14 days, and were used to develop this new chick memory model. Experimental procedures were approved by the University Complutense of Madrid and in compliance with the Spanish Guidelines for the Care and Use of Animals in Research. The experimental model consisted of a 14-day trial with 3 different phases: pre-training (from 2 to 9 days old), training (at 10 days old) and test (from 11 to 14 days old). At arrival every chick was tagged and placed in their respective pen. During the first week (from 2 to 9 days old), birds were daily pre-trained: chicks from each pen were placed together in a separate cage and after 5 minutes of acclimatation two 5 mm diameter beads (chrome and blue) were offered for 2 minutes. Their response (actively pecked or not) was daily monitored. Only those birds showing, every day, high interest pecking behavior were included in the training and test phases. Accordingly, approximately 40% of chicks were excluded from the trial. The training phase was conducted in 10 days old chicks and consisted of a single presentation for 1 minute of a 5 mm bright chrome bead dipped with the strong aversive methyl anthranilate. Chicks that peck the bead dipped in methyl anthranilate showed a reaction of disgust (backing away, shaking their heads, and wiping their bills) as was previously described by other authors (Rose 2000). The capacity to remember this distasteful substance, was monitored during the following 3 days in the test trial. For that, trained birds were offered the blue and chromed (without aversive) beads and those chicks that pecked the blue bead avoiding pecking the chrome one were considered as “birds that retained the information” and the percentage of those bird were calculated. Our results showed that 24 hours after training almost 90% of the chicks retained the information. The retention rate decreased along the time to reach 36% after 72 hours. In conclusion, the memory model developed permitted the training of more than 60% of chickens within a week and allows to evaluate the effect of nutritional interventions on memory and cognitive functions.

P 164 ASSESSEMENT OF ANTHOCYANIN-RICH EDIBLE FLOWERS BIOACCESSIBILITY USING FOOD PROCESSING APPROACHES

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The role of diet in human health remains one of the main concerns of modern society. New strategies to fulfill the necessity of healthy diets available to everyone, in a growing civilization, with increasing awareness of the consumers, and the need for more sustainable agriculture strategies to reduce climate and water footprints, led to an increase of research focused on such themes, and some forgotten traditions are being reinvented and adopted. In this perspective, edible flowers (EF), are gaining more and more popularity among consumers, chefs and confectioners, and although they still represent a niche market, EF constitute an emerging and sustainable dietary alternative. Amidst EF, there are some very good sources of such bioactive components, in particular, polyglycosylated ANT that have been reported as having better physical and chemical properties than monoglysocylated ANT [1]. Although a few health-benefits of ANT-rich EF have been reported, no knowledge about their bioavailability is at hand.

In this work, four species of Edible Flowers (*Viola tricolor L.*; *Centaurea cyanus*; *Cosmos bipinnatus*; *Clitoria ternatea*) – Figure 1 – were characterized in terms of anthocyanin content and for their stability towards different food processing approaches. Anthocyanin-rich extracts were submitted to different cooking temperatures and times and the degradation kinetics of the total anthocyanins was followed by UPLC-PDA. Also, the pH influence was assessed using the same methods. The influence of different food matrices elements including proteins, starch, and sugars, in the stability of anthocyanins to the cooking procedures was also assessed. And finally, the absorption of the resultant samples through an *in vitro* gastric cell model was evaluated using caco-2 cells.

The results showed that the anthocyanins were mainly polyglycosylated. Temperature and pH had a significant effect on the degradation rate of anthocyanins. However, in some cases, a time-dependent rise in the amount of the total anthocyanin content in similar conditions of T and pH was observed. Different effects dependent of the food matrix element, were observed. Overall, all the anthocyanins regardless of the source were able to be detected on the basolateral side of the gastric cell model, indicating their ability to be absorbed and cross it.

This exploratory study showed the first insights on how cooking can influence the bioaccessibility and bioavailability of anthocyanins from edible flowers.

Figure 1: Edible Flowers utilized in the different experiments.

[1] He, J., et al., *Comprehensive Reviews in Food Science and Food Safety*, 2022. **21**(4): p. 3096-3128.

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P 165 CONSUMPTION OF ELLAGITANNINS-RICH JABUTICABA POWDER FOR 21 DAYS INCREASES UROLITHIN EXCRETION IN NORMOWEIGHT PEOPLE AND MODIFIES METABOTYPES BY TURNING NON-PRODUCERS INTO UROLITHINS PRODUCERS

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The stratification of individuals into metabotypes in clinical studies becomes increasingly important to observe the effect of a health intervention. Jabuticaba is a Brazilian berry which peel and seeds contain a high content of ellagitannins. The aim of this study was to evaluate if the consumption of jabuticaba peel and seed powder (JPSP) for 3 weeks could modify the initial characterization of the ellagitannins metabotype (A, B or 0) and the degree of urolithin excretion. For the clinical study, adult volunteers (n=59) of both sexes, with a body mass index (BMI) between 18.5 and 40.0 kg/m^2 were recruited. The study consisted of the daily consumption of 4 capsules containing either the placebo (2 g of corn starch) or JPSP (3 g containing 35.2 mg of vescalagin + ellagic acid) for three different periods of 21 days, in the following order: 1) placebo consumption; 2) JPSP consumption; 3) placebo consumption. To verify possible changes in individuals' metabotypes and amount of urolithins excretion, volunteers acutely ingested 3 g of JPSP right before the beginning of each period and after the last period. Urine samples (period between 24 h and 36 h after ingestion) were collected before and after all interventions for the analysis of the metabotype and degree of excretion of urolithins. Considering all volunteers (n=59), the metabotypes distribution was 32.2% of metabotype A (UM-A), 52.5% of metabotype B (UM-B), and 15.3% of metabotype 0 (UM-0), which differed from those already reported in the literature in different populations. No statistical difference was found between sexes regarding UM-A and UM-B distribution. Considering BMI, normoweight (NW) volunteers presented a higher prevalence of UM-B (p=0.04) than UM-A. In contrast, overweight/obese (OW/OB) volunteers presented a similar distribution of UM-A (43%) and UM-B (40%). We observed that with increasing age, there was an increase in the % of UM-B and a decrease in the % UM-A. After JPSP consumption, six metabolites were identified in urine: urolithin A 3/8-glucuronide (Uro-A 3/8-glu), isourolithin A 3-glucuronide (IsoUro-A 3-glu), isourolithin A 9-glucuronide (IsoUro-A 9-glu), isourolithin A (IsoUro-A), urolithin B-glucuronide (Uro-B-glu), and urolithin B (Uro-B). The glucuronidated forms were the most abundant metabolites, representing 93–99% of the total excreted at all stages of the study and showed high variability (0.06 µmol to 15.39 µmol). Looking at the changes in excretion between periods of the study we found no significant differences when volunteers were separated by sex or body fat. When volunteers were classified according to BMI we found that the JPSP intervention led to an increase (p=0.04) in the excretion of urolithins by NW volunteers, whereas the excretion by OW/OB volunteers did not change. Moreover, before the intervention with JPSP, nine individuals were characterized as UM-0, since no urolithins were detected in the urine. After JPSP intervention four of them started to excrete detectable amounts of urolithins in the urine, demonstrating that it was possible to modify ellagitannins metabotypes upon a 21-days exposure to a food source of these bioactive compounds.

P 166 NUTRIGENETICS – HOW GENETIC VARIATION MODULATES INFLAMMATORY RESPONSE TO DIETARY FIBER CONSUMPTION

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It is recognized that a diet associated with positive health outcomes encompasses consumption of dietary fibre (DF), present in fruits and vegetables, among others. However, dietary constituents do not affect all individuals equally. As it has been found that the gut microbiota plays a significant role in immunomodulation of their host, the host-microbiota interface gained relevance in understanding the development of many non-communicable chronic conditions, including cardiovascular disease, cancer, autoimmunity and neurodegeneration. In this sense, DF is an important link between gut microbiota and host inflammatory profile. DF is fermented by microbial species in the gut, and absorbed by the host as short-chain fatty acids (SCFA), presenting immune and metabolic modulating functions across the human body. Factors that might influence the fermentative capacity of the microbiota (e.g. age, birth mode, medication intake, alcohol and tobacco consumption, pathogen exposure and physical activity) may reveal clues on SCFA production capacity and bioavailability, apart from the SLAMENGHI factors (i.e., molecular species, linkage, amount, matrix, effectors of absorption, nutrition status, genetics, host-related factors, and the interaction of these). Indeed, host genetic background also modulates bacterial colonization.

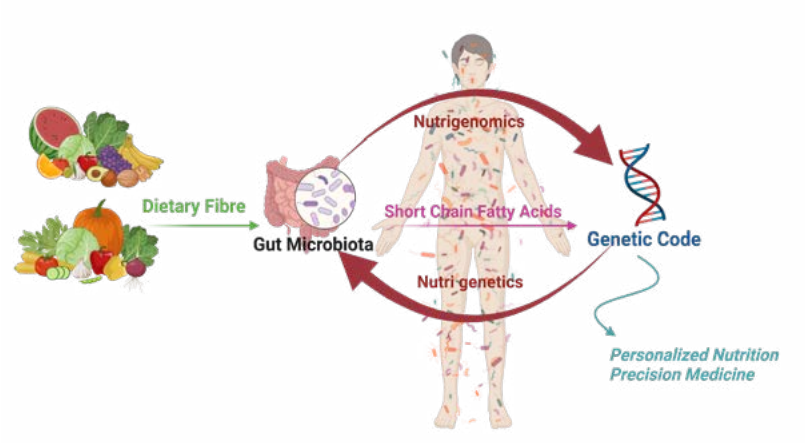
While SCFA (acetate, propionate, butyrate) were significantly associated with health beneficial effects in *in vitro* studies, results *in vivo* are not consensual. For example, butyrate arrests proliferation of colon cancer cells via the Warburg effect, however it is essential for proliferation and differentiation of healthy colonocytes. Furthermore, SCFA metabolic pathways are not fully understood.

Nutrigenetics focuses on how individual genetic profiles, copy number variation (CNVs) and single nucleotide polymorphisms (SNPs) may influence fates of different food items through, e.g. absorption, distribution, metabolism or excretion (ADME) patterns. In recent years, CNVs and SNPs in coding and non-coding regions of the genome were identified as drivers of phenotypical differences among individuals. Polygenic risk scores have been associated with phenotypes across various human pathologies.

When describing homeostasis on the holobiont level (this is, when taking the host genome and microbiome together), polygenic risk is thus relevant to predict the impact of SCFA in individual subjects. On the other hand, absorbed SCFA can have nutrigenomic effects, i.e. leading to altered gene expression in specific cells and tissues.

In this presentation we present the state-of-the-art understanding of several strata leading to inter-individual variability, enterotypes, genotypes and lifestyle, and later focus on identifying possible sources of genetic variation such as GPR41, GPR43 or GPR109A (G-protein coupled receptors for SCFA), transporter genes of the SLC16A family (monocarbohydrate transporters), effector genes such as MUC2 (for mucus layer production in the colon) or regulatory genes such as NRF2, that may be indicative of the phenotypic flexibility in response to diet, particularly DF.

FIG 1: TOWARDS PRECISION NUTRITION.



This complex interplay may allow for the development of predictive models on the life/long-term adaptations to DF, such as maladaptation and tissue damage, which may develop into disease in individuals with specific predispositions. These insights allow the development of better personalized prediction, and greater individual care with respect to supportive therapeutic approaches and potential health effects, such as following intervention with DF.

P 167 BIOACCESSIBILITY AND BIOAVAILABILITY OF PHENOLIC COMPOUNDS IN GALICIAN VIRGIN OLIVE OILS

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The Mediterranean Diet is a complex dietary pattern that includes high consumption of nuts, fruits, vegetables, legumes, whole-wheat products, fish, and red wine and whose main lipid source is virgin olive oil (VOO). Despite the well-described beneficial effects associated with VOO consumption, it is still necessary to deepen the bioaccessibility and bioavailability of the bioactive compounds (such as, phenolic compounds) since the biological role of them in human organisms is attributed to their metabolites.

The aim of this work was to evaluate the bioaccessibility of VOO phenolic compounds by using the INFOGEST standardised *in vitro* gastrointestinal method and the components of bioavailability by using Caco-2 cells (clone HTB-3). The stability of the phenolic fraction before and after *in vitro* digestion were studied by using HPLC with several detectors: diode array detection (DAD), fluorescence (FLD) and high resolution tandem-mass spectrometry (MS/MS). Furthermore, the bioaccessible VOO-related phenolic compounds and their metabolites were tracked during a simulated transepithelial transport *in vitro* by UHPLC nano LC system coupled to a QExactive Orbitrap MS at different concentrations (5, 25 and 50 µg/g).

The INFOGEST *in vitro* digestion model involves three steps (oral, gastric, and intestinal digestion) and generates two fractions after each one: an aqueous fraction (Wp) and an oily fraction (Op). The results showed that secoiridoids were the most abundant family in the VOO polar fraction (98% of the total phenolic compounds). After oral digestion, the distribution of phenolic compounds between the two fractions was determined by their polarity: phenolic acids and simple phenols (hydroxytyrosol, hydroxytyrosol acetate and tyrosol) were mainly detected in Wp, meanwhile flavonoids (apigenin, diosmetin and luteolin) and lignans were mostly found in Op. After gastric digestion, large hydrolysis of secoiridoids was observed to generate simple phenols. The instability of secoiridoids after intestinal digestion was again responsible for the release of simple phenols which were mainly recovered in Wp together with flavonoids. Conversely, lignans were stable to intestinal conditions and remained in Op.

The intestinal uptake of VOO metabolites has been determined in the basolateral (BL) side after different times (30, 60, 120 and 180 minutes). Phenolic compounds bioavailability was found to be concentration-dependant. Namely, these results showed that tyrosol was the most bioavailable compound after treating with the lowest concentration (5 µg/g), although hydroxytyrosol and their metabolites (homovanillic acid and homovanillic alcohol) were also determined in the BL side. Likewise, a high proportion of hydroxytyrosol-3'-sulphate at medium and highest concentration (25 and 50 µg/g) was also detected in the BL side, indicating a very efficient sulphation by Caco-2 cells and a preferential permeation of sulphation metabolites to cross to the BL compartment. Moreover, other hydroxytyrosol metabolites together with tyrosol and apigenin were determined in the BL side. These results have made it possible to monitor, visualize and reveal metabolism of the phenolic compounds in Galician VOOs. An extensive metabolism of phenolic compounds from VOO were observed in the *in vitro* intestinal model favouring the absorption and permeation process. Further studies are required to correlate these findings to the health-related effects.

P 168 *IN-VITRO* DIGESTION OF NATIVE MILK FAT GLOBULES FRACTIONS AFTER THE MICROFILTRATION PROCESS

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Milk fat occurs in the form of milk fat globules with sizes ranging from 0.2 to 15 µm. They are characterized by a complex and organized structure, where their interior is made up of a triglyceride core surrounded by the fat globule membrane. This membrane protects the globules mainly against coalescence and lipolytic activity. The quality and properties of this interface constituted by phospholipids and proteins have a significant impact on the availability of triacylglycerols for lipase. Many investigations point to a complex mechanism of the lipolysis process and to the significant role of the size of fat globules and, consequently, of the available interface in the process of digestion. Therefore, the aim of this study was to compare the digestive properties of the fractions of native milk fat globules obtained after the microfiltration process. This method allows to obtain milk fat globules fractions of different sizes.

The research material was raw cow's milk, which was subjected to the microfiltration process. The obtained retentate, permeate and milk fraction were subjected to *in-vitro* digestion under the influence of lipase and the amount of released fatty acids over time and changes in the emulsion system were determined.

The fractions obtained after the microfiltration process differed in both the fat content and the size of the fat globules. Retentate contained the most fat and the largest globules, the permeate contained the least fat and small globules. Digestion of fat globules was the fastest in the permeate containing the smallest globules, and the slowest in the permeate. These studies confirm the dependence of the rate of milk fat digestion on the size of the globules and the available interfacial surface. The speed of the digestion process was also confirmed by the intensity of changes in the emulsion system.

The obtained results indicate the important role of the available interface in the process of milk fat digestion under the influence of lipase. In addition, they indicate the possibility of using membrane filtration processes to obtain milk fractions showing different nutritional properties and thus using them in the design of food with specific functional features, differing in the rate of release of ingredients or digestibility.

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P 169 GASTROINTESTINAL STABILITY AND INTESTINAL ABSORPTION OF BIOACTIVE PEPTIDES IDENTIFIED IN POULTRY BY-PRODUCT HYDROLYSATE

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Bioactive peptides produced by enzymatic hydrolysis of different food proteins, including side-streams of meat processing, have been reported in the last decades. For example, peptides with antidiabetic, antihypertensive, anti-inflammatory, antioxidant, hepatoprotective and lipid regulatory activities were identified in poultry hydrolysates. It is considered that hydrolysates with bioactive peptides have potential as nutraceuticals. Such nutraceuticals can prevent or delay the onset of lifestyle-related diseases such as diabetes type II or hypertension. Therefore, bioavailability, absorption, distribution, metabolism, and excretion of bioactive peptides have to be evaluated. In this study, we focused on gastrointestinal stability and absorption of bioactive peptides. Our objectives were to evaluate gastrointestinal stability of angiotensin converting enzyme-1 (ACE-1) and dipeptidyl peptidase IV (DPP4) inhibitory peptides and to study transport of these bioactive peptides across intestinal epithelia.

Methods:

Enzymatic protein hydrolysis was used to produce a hydrolysate with bioactive peptides from mechanically deboned chicken residues. Bioactive peptides were identified with liquid chromatography tandem mass spectrometry (LC-MS/MS) in the most potent fraction produced by bioactivity-guided fractionation. Evaluation of ACE-1 and DPP4 inhibition of the hydrolysate's fractions were performed using assay kits. Fractionation was performed using HPLC system with size exclusion chromatography (SEC) and reverse phase columns. Identified dipeptides were verified by testing chemically synthesized dipeptides.

Gastrointestinal stability of the peptides was evaluated using standardized international consensus INFOGEST static model. SEC fraction with the average molecular weight of 514 Da that contained bioactive peptides were produced in larger quantities to perform *in vitro* digestion. Stock concentration of the SEC fraction was 50 mg/mL.

Absorption of peptides were studied using Caco-2 cell monolayers. The transport experiment was performed in Dulbecco's Modified Eagle Medium (DMEM). Transepithelial transport of the bioactive SEC fraction (1 mg/mL and 100 µg/mL) and two pure dipeptides (100 µg/mL and 10 µg/mL) were evaluated. The integrity of Caco-2 monolayers was monitored by transepithelial electrical resistance (TEER) measurements and fluorescein isothiocyanate dextran (FITC-d) assay. Identification of peptides after *in vitro* digestion and absorption studies were performed using LC-MS/MS.

Results:

Several dipeptides with ACE-1 and DPP4 inhibitory activities were identified in the most potent fractions of the hydrolysate.

Identified dipeptides were detected after *in vitro* digestion indicating that they are resistant to the proteases of the gastrointestinal tract.

TEER measurements and FITC-d assay demonstrated that Caco-2 monolayers were not disturbed by 1 mg/mL of SEC fraction or 100 µg/mL of pure dipeptides. LC-MS based identification of bioactive peptides after transepithelial transport is currently being analyzed.

Conclusion:

Our study shows that ACE-1 and DPP4 inhibitory dipeptides identified from poultry by-product hydrolysate are resistant to gastrointestinal digestion. Transport of these bioactive dipeptides across intestinal epithelia is under investigation. First results show that 1 mg/mL of SEC fraction and 100 µg/mL of pure dipeptides have not disrupted Caco-2 monolayers.

P 170 THE INTERINDIVIDUAL VARIABILITY IN FLAVAN-3-OL METABOLISM

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Recently, the very first *dietary bioactive guideline* was published, recommending 400–600 mg/d for cardiometabolic protection of flavan-3-ols, a subclass of polyphenols primarily found in tea, chocolate and some fruits. However, huge interindividual differences in clinical effects have been observed, likely due to variation in the amount and types of metabolites formed during digestion. Although the reasons for the variability are not fully understood, previous studies have proposed that differences in the metabolism of colonic microbiota among individuals could play a role. This study aims to investigate this hypothesis.

17 healthy Caucasian males ingested a single dose of 150 mg (–)-epicatechin, a flavan-3-ol monomer. Blood samples were taken every hour for 14 h, as well as at 24 h and 48 h after intake. Using UHPLC-HRMS, plasma samples were analysed for small intestine absorbed epicatechin metabolites (SREM) and phenyl-γ-valerolactones and phenylvaleric acids (PVLs and PVAs), the main metabolites produced by the gut microbiome. A stool sample obtained the day before intake was analysed using quantitative microbiome profiling (flow cytometry and 16S-rRNA).

SREM peaked at 2.8 ± 1.4 h, while PVL and PVA peaked at 10.5 ± 2.2 h. The results showed pronounced interindividual differences in the plasma levels of individual SREM, PVLs and PVAs metabolites, with some producing very limited amounts of specific metabolites, while others produced higher amounts. The coefficient of variation was 82, 49 and 69 % for the sum of all SREM, PVL and PVA, respectively. Higher amounts of PVL were correlated with higher levels of PVA ($r=0.610$; $p=0.009$), while, interestingly, the amount of SREM also seemed positively associated with the circulating levels of PVL and PVA combined ($r=0.480$; $p=0.051$). Quantitative microbiome profiling (QMP) confirmed the interindividual differences in microbial composition in terms of taxa composition and absolute abundance. Some SCFAs-producing amplicon sequence variants like Lachnospiraceae, Ruminococcus, and Roseburia were positively correlated to the number of microbial metabolites.

Overall, the bioavailability of SREM, PVL, and PVA showed significant interindividual variability, even in this homogenous sample of young healthy males. Notably, clear correlations were found between (epi)catechin-metabolizing bacteria and SCFAs-producing bacteria and the metabolic pathway of (–)-epicatechin, which serve as important explanatory variables for interindividual differences in pharmacokinetic profiles. Along with microbiome-related features, other characteristics, such as habitual flavan-3-ol intake, may contribute to this variation, ultimately affecting the health outcomes of flavan-3-ol consumption. Further investigation into the causes of such interindividual variability can help to optimize the health benefits of these compounds.

P 171 *IN VITRO* STUDY: HEPATIC GLUCURONIDATION OF (+)-CATECHIN AND (-)-EPICATECHIN IN HUMANS AND RATS

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Rich in fruits and vegetables, olive oil, fish and legumes, the Mediterranean diet shows promise for healthy aging, reducing risk of major chronic diseases (1). Some nutrients of this diet, like polyphenols, are particularly beneficial for health. Polyphenols are secondary metabolites, produced by many plant species. There are 4 classes: flavonoids, stilbenes, phenolic acids and lignans (2). Their effects on health are widely described (3): the biological activities of polyphenols largely depend on their bioavailability. Once ingested, nutrients achieve the digestive system where they can be absorbed through the intestinal barrier, metabolized in the intestine, by microbiota and in the liver, before reaching tissues and organs. Studies of metabolism are therefore crucial to understand which molecules are responsible for biological effects. Among metabolic reactions, glucuronidation is one of the main reactions of phase II metabolism. A glucuronic acid is added to a parent molecule by glucuronotransferases, enzymes located in the endoplasmic reticulum of most tissues, particularly the liver.

This work aimed to characterize, *in vitro,* the hepatic glucuronidation of two flavonoids, (+)-catechin and (-)-epicatechin, contained in active plant ingredients produced by Activ'Inside, such as Memophenol™ (a patented formula rich in polyphenols). Liver protein extracts from humans and rats and an UHPLC-MS/MS approach will be used.

The first results showed that (+)-catechin and (-)-epicatechin can be glucuronidated by rat and human liver protein extracts. Analysis by UHPLC-MS/MS revealed the formation of four monoglucuronide metabolites for each flavonoid, in both rats and humans. No diglucuronide was observed. However, interspecies differences were found in terms of the quantity of monoglucuronides formed. Indeed, in rats, two of the four glucuronides are predominant for each flavonoid whereas, in humans, a single monoglucuronide accounts for the majority. Moreover, the metabolism in animals seems to be more important than in humans since we have found more monoglucuronide metabolites formed in rats for the same experimental conditions.

In this study, we identified, *in vitro*, the formation of four monoglucuronide metabolites from (+)-catechin or (-)-epicatechin, by human and rat liver protein extracts. Their structure (i.e. the place of the glucuronide on the molecule) will be then characterized by NMR after production and purification by semi-preparative HPLC of larger quantities of these metabolites. These compounds produced, not yet commercialized, could be used as standards to be quantified in biological samples from (pre) clinical studies.

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P 172 CYTOTOXIC EFFECT OF PHENOLIC METABOLITES FROM OLIVE LEAVES AGAINST PANCREATIC CANCER CELL LINES

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Phenolic compounds present in plants have been shown to have beneficial properties for the treatment of different chronic diseases, including cancer[MC1] [1]. However, it should be considered that the phenolic compounds are heavily metabolized by humans and, in most cases, the resulting metabolites are actually responsible for a large part of the observed effects, since they modulate multiple metabolic pathways and have mechanisms of action on gene expression [2]. For that purpose, it is important to evaluate both the parent compounds, as well as their metabolites, since these are the compounds present in the blood circulation.

Different metabolites detected in the bloodstream of human after accute ingestion of a supplement based on olive leaf (*Olea europaea*) extract were previously annotated at different times. Among them, oleuropein, hydroxytyrosol, hydroxytyrosol glucuronide, dihydro-ferulic acid and its sulphate and glucuronide forms, as well as homovanillic acid and vanillic acid and their derived sulphate metabolites, among others, were found.

Given the urgent need for new effective treatments for pancreatic cancer, we conducted a preliminary cytotoxicity study using the MTT assay to evaluate the anticancer effect of phenolic compounds present in olive leaf and their respective metabolites in human pancreatic cancer cells (Panc-1 and MIA PaCa-2 cells).

After incubation of the cell lines for 24, 48 and 72 h at concentrations of 5, 10 and 20 µM of the different metabolites present in the olive leaves, we observed that the most potent compounds that reduced cancer cell growth were dihydroferulic acid sulphate and vanillic acid sulphate, followed by homovanillic acid sulphate. This was observed in both MIA PaCa-2 and Panc-1 cells.

In conclusion, these preliminary studies suggest that sulphated dihydroferulic acid, sulphated vanillic acid and sulphated homovanillic acid, which are metabolites derived from phenolic compounds originally present in olive leaf, deserve further evaluation for their anticancer effects.

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P 173 A SYSTEMATIC REVIEW ON (POLY)PHENOL – GUT MICROBIOME INTERACTIONS AND HUMAN HEALTH

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The gut microbiota is now recognised as fundamental to human physiology and a determinant of host health and disease risk. The gut microbiota has been shown to regulate host immune and metabolic processes involved in chronic diet and life-style related non-communicable diseases, such as obesity, diabetes, cardiovascular disease, dementia, and autoimmune diseases. On the other hand, plant-based foods are rich and exclusive sources of a wide range of bioactive phytochemicals, especially (poly)phenols. Dietary (poly)phenols undergo an intensive metabolism, mainly at the colonic level, generating a plethora of potentially bioactive catabolites, which have been recognised to be involved in the protective effects from many non-communicable diseases associated with consumption of (poly)phenol-rich sources.

Based on these observations, this systematic review aims to elucidate the influence of gut microbiota-derived (poly)phenol catabolites and/or (poly)phenol-mediated gut microbiome modulation on human health biomarkers related to inflammation, neuroprotection, cardiometabolic and immune function.

A literature search was performed using PubMed, Scopus, Web of Science, Medline via Ovid and EMBASE, limited to English language human intervention studies published between 1995 and February 2021. Two independent reviewers performed data extraction, and disagreements were resolved through consensus.

A few thousand studies were identified from the database search. After removing duplicated articles and articles which did not meet the inclusion criteria, 61 human intervention studies were included.

A few papers reported health effects due to gut microbiota modulation following (poly)phenol intervention. These papers dealt with cardiometabolic outcomes, and only two studies reported a significant improvement in energy expenditure and vascular reactivity together with a significant modulation of gut microbiota composition.

Most of the selected papers investigated the role of (poly)phenol gut catabolites on health. The main significant results were related to cardiometabolic status improvement, mainly obtained after isoflavone or flavan-3-ols consumption, and measured a positive modulation of total, LDL and HDL cholesterol, triacylglycerol level, blood pressure, vascular function, expression of genes associated with energy metabolism and inflammatory processes, fat mass, peptide YY, uric acid and alanine transaminase, always in the presence of specific gut (poly)phenol catabolites.

The mutual effect of (poly)phenol catabolites and gut microbiome on human health has been addressed in 19 papers. Most of the studies demonstrated that the consumption of (poly)phenols enhanced the circulating level of gut-derived catabolites, and induced microbiota modulation, but not in many cases positively and significantly impacted those parameters generally associated with cardiometabolic issues, including blood pressure, lipid profile, glycemia, vascular function, body weight and BMI, or improved the inflammatory status.

In conclusion, limited evidence emerged for (poly)phenol – gut microbiome – immune-, inflammation- and neurofunction interaction. More substantial evidence supported the role of (poly)phenol or gut microbiome alone and their mutual interplay on cardiometabolic outcomes. However, more efforts are needed to perform properly designed studies employing specifically tailored analysis to simultaneously elucidate the *in vivo* circulating (poly)phenol catabolites, the gut microbiota modulation and the possible derived health effect, also taking into account the inter-subject variability.

P 174 *IN VITRO* COLONIC FERMENTATION OF PLANT STEROL-ENRICHED WHOLEMEAL RYE BREAD MODULATES INTESTINAL MICROBIOTA

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It has been demonstrated that plant sterols (PS) have a modulating effect on the microbiota increasing the Firmicutes phylum after an *in vitro* colonic fermentation of an ingredient source of PS[1]. An increase in Bacteroidetes phylum has also been indicated after an *in vitro* colonic fermentation of PS-enriched milk-based fruit beverages[2]. In addition, fiber consumption can modulate intestinal microbiota as well, favoring the growth of some health-promoting microbial species (*Lactobacillus* and/or *Bifidobacterium*)[3]. The aim of this study was, for the first time, to assess the effect of a high-fiber (13.7 g/100 g) wholemeal rye bread enriched with PS (2.3 g/100 g) on intestinal microbiota composition. An *in vitro* fermentation assay was carried out using the dynamic multi-compartmental digestion system simgi® (CIAL-CSIC). The system was fed daily with 80 g of bread during five days. Samples of fermentation liquids from ascending, transverse, and descending colon compartments (AC, TC and DC, respectively) were taken at 0, 24, 48, 72, 96 and 120 h. For microbiota composition analysis, the V3-V4 region within the 16S rRNA gene was sequenced by PCR amplification. In AC the abundance of *Lactobacillus* increased over the fermentation time from 1.5% to 99.4%, while *Escherichia shigella* decreased from 81.5% to 0.6%, both reaching a plateau at 72 h. In the distal colon compartments (TC and DC), a higher diversity of microbiota composition was observed at 0 h: *Bacteroides* (22.8 and 19.1%), *Parabacteroides* (24.6 and 18.3%), members of the Lachnospiraceae family (19.5 and 21.3%) and *Escherichia shigella* (14.8 and 23.0%). *Bacteroides* and *Parabacteroides* decreased to an abundance of less than 0.3% from 48 h onwards in TC, while in DC these reached their minimums (0.04%) at 120 h. The abundance of Lachnospiraceae family decreased during the fermentation assay (from 9.7–11.7% to 0.03–1.8%) with a concomitant increased of *Lactobacillus* up to 93.5–97.4% at 120 h. *Escherichia shigella* decreased until 0.4–0.6% similar to AC. Moreover, in TC and DC *Bifidobacterium* showed maximum values at 48 h (30.1 and 10.4%, respectively), while the abundance of *Bilophila* was below 5% in both compartments. *Akkermansia* was only detected in DC with an initial and final abundance of 18.8 and 0.02%, respectively. The *in vitro* fermentation of PS-enriched wholemeal rye bread produces a higher diversity of microbial species in the distal colon compartments (TC and DC) than in the AC. Furthermore, the modulation of microbiota composition during fermentation time results in increased abundance of *Lactobacillus* and *Bifidobacterium*, which may have a prebiotic effect.

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Acknowledgements:

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P 175 INDUSTRIAL AND CULINARY TREATMENTS IMPACT ON THE GUT MICROBIOTA CATABOLISM OF (POLY)PHENOLIC COMPOUNDS OF PIQUILLO PEPPER (*Capsicum annuum* CV. PIQUILLO)

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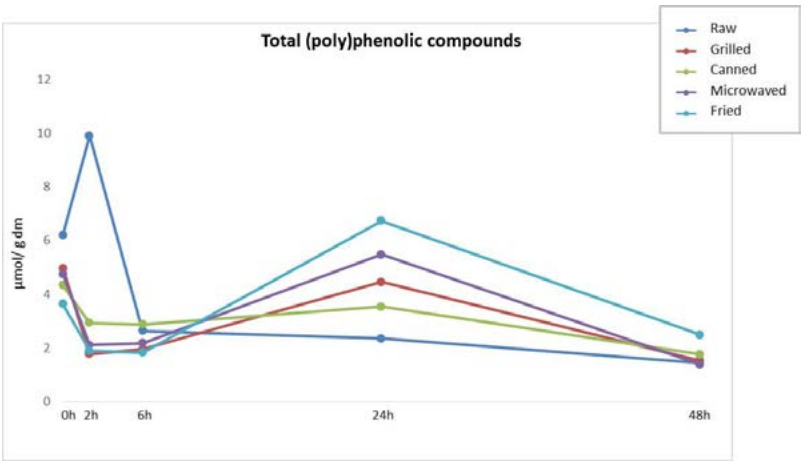
Piquillo pepper (*Capsicum annuum*) is a highly consumed gastronomical product of the Mediterranean diet. It is a source of (poly)phenols, bioactive compounds with potential health effects on lowering the incidence of several chronic diseases. Besides giving organoleptic attributes, the industrial heat treatments established by the Protected Designation of Origin, also impact on its (poly)phenolic profile (Del Burgo-Gutiérrez et al., 2023) which might influence their absorption and metabolization by the colonic microbiota and therefore, their bioactivity. The present study aimed to investigate the impact of industrial (grilling and canning) and culinary processes (microwaving or frying) on the *in vitro* catabolism of (poly)phenolic compounds by the action of gut microbiota.

Raw and heat-treated Piquillo peppers were subjected to a simulated gastrointestinal digestion and to a 48 hours *in vitro* colonic fermentation using faeces from 3 healthy donors who followed a 2-days low-polyphenol diet (Ethical approval from the Research Ethics Committee of University of Navarra nº 2021.80 TESIS). Samples were collected at different times of incubation (0, 2, 6, 24 and 48 hours). (Poly)phenols were extracted with methanol/acidified water (0.1% formic acid) (50:50 v/v) and analysed by HPLC-ESI-MS/MS.

A total of 52 (poly)phenols were identified in Piquillo pepper samples after *in vitro* colonic fermentation, from which 6 compounds derived from the microbial catabolism of native (poly)phenols of Piquillo pepper. In raw pepper, total (poly)phenols increased after 2 hours of incubation (from 6.219 to 9.906 µmol/g), mainly due to the rise of flavonoids (from 2.584 to 5.7 µmol/g). Nevertheless, after 6 hours total (poly)phenols decreased constantly, suggesting that (poly)phenols in raw pepper are covalently bound to food matrix and released at the initial steps of colonic fermentation to be further catabolized.

In heat-treated peppers, (poly)phenols seemed to be released during processing and gastrointestinal digestion enhancing their metabolization by colonic microbiota into low molecular weight (poly)phenol derivatives after 24 hours of incubation, particularly into 3-(3'hydroxyphenyl)propionic acid, 1,2-benzenediol and 4'hydroxy-3'-methoxyphenylacetic acid. Interestingly, although fried peppers exhibited the lowest (poly)phenolic content at baseline (0 h), after 24 hours of incubation presented the highest concentrations (6.719 µmol/g) suggesting that the addition of oil might protect against gastrointestinal degradation by trapping (poly)phenols into lipid micelles and also delaying its colonic catabolism.

Figure 1: Total (poly)phenolic compounds during 48 h of colonic fermentation.



Overall, the poly)phenolic profile modification as result of the application of industrial treatments influences their metabolization by the colonic microbiota. Moreover thermal treatments, specially frying, enhance (poly)phenols metabolization leading to the formation of higher amounts of lower molecular weight compounds that would be later absorbed into blood stream to exert their potential health benefits.

Financial support:

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Reference:

Del Burgo-Gutierrez et al., (2023) *J. Agric. Food Chem*. 71, 15, 6050–6060. DOI:10.1021/acs.jafc.2c07829.

P 176 EFFECTS OF PLANT-BASED DIET ON GUT MICROBIOME COMPOSITION, HOST METABOLOME AND MICROBIOME RESILIENCE IN THE CONTEXT OF *Clostridioides difficile* INFECTION

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Background and Objectives:

Gut microbiome resilience is the restorative capacity of the microbiome following a perturbative event (e.g. antibiotics or unhealthy diet). Healthy diets (e.g. Mediterranean) are inversely correlated with chronic disease risk, being high in fruit and vegetables, rich in fibre, low in simple sugars and saturated fat, in contrast to the westernised diet, high in saturated fat and refined foods. Impairment of the gut microbiome allows infection by opportunistic pathogens (pathobionts) e.g. *C. difficile*, a major cause of healthcare-associated infection. The colonic milieu reflects metabolic outputs of specific dietary patterns (e.g. (poly)phenols and SCFA), faecal water (FW) is therefore of physiological relevance as it is the aqueous phase of faeces. The effects of westernized (W) and plant-based (PB) diets on host metabolome and microbiome composition were investigated. Subsequently FW derived from both dietary patterns was assessed to determine its impact on *C. difficile* biology.

Methods:

A single-blind, randomised, controlled two-way crossover dietary intervention study (Clinical Trials.gov NCT05231317) was conducted in 11 participants (BMI > 18, aged 18 - 70, free-living, non-smokers) which consumed two diets: PB diet (polyphenol rich) and W diet (high saturated fat, high in processed foods) for 14 consecutive days, separated by a 7-weeks washout period. Participants were sampled (blood, urine, faeces) pre and post intervention periods. Metabolites concentrations were evaluated *via* NMR in plasma, urine, and faecal water. Plasmatic bile acids concentrations were evaluated with UHPLC-MS/MS and (poly)phenols in urine *via* UHPLC-HRMS. Microbiota composition was analysed via 16S rRNA amplicon sequencing. The best responder to the PB diet was selected and FW growth media (day 1 & 14) prepared for both intervention periods. *C. difficile* 630 was then cultured with these FW enriched growth media and cell pellets harvested (mid-exponential log growth phase), RNA extracted, and transcriptomic analysis (RNA-seq) was performed on *C. difficile* 630 to determine the differential effect of diet on *C. difficile* biology.

Results:

PB diet displayed significant reduction in plasmatic total and primary bile acids compared to the W diet. PB diet significantly increased urinary (poly)phenolic compounds: m-coumaric acid, 3-(3′-Methoxy-4′-hydroxyphenyl)propanoic acid, 3-(4′-Methoxy-3′-hydroxyphenyl) propanoic acid, 4′-hydroxymandelic acid). Urinary creatine, associated with meat consumption decreased significantly. PB diet had limited impact on the gut microbiota with significant reduction in *Subdoligranulum* genus only. Preliminary analysis of *C. difficile* transcriptomes from bacterial cells cultured in FW from PB & W diets (day 14), revealed significant global changes in gene expression involving transport binding proteins and lipoproteins (12.9% of total), metabolism of amino acids (7.6%), glycolytic pathways (4.2%) and sporulation genes transcription (2.4%). PB diet had a differential effect on implied *C. difficile* pathogenicity (sporulation) compared to the W diet, significant decreases ($p<0.001$) were observed in the master regulator *spo0A*, genes involved in stage II sporulation (e.g *spoIIGA*, *spoIIE*), stage V (e.g. *spoVAD*), while the expression of genes encoding spore coat proteins (CotA, CotE and stage V proteins) were significantly increased ($p<0.001$).

Conclusions:

The data suggest that diets rich in fruits and vegetables can beneficially alter the pathobiology of the opportunistic human pathogen *C. difficile*.

P 177 NEW FINDINGS IN THE METABOLISM AND INTERACTION OF SAFFRON CAROTENOIDS WITH GUT MICROBIOTA

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Background

Crocins and crocetin are natural carotenoid-type compounds found in the stigmas of saffron flowers (*Crocus sativus L.*). Numerous biomedical and pharmacological properties have been attributed to these compounds, mostly those related to the effects on the central nervous system with action on memory and learning, neurodegenerative diseases, depression and anxiety, among others. However, the molecules or metabolites responsible for these effects are still unknown. Recent studies with mice point to the crucial role of intestinal microbiota in the metabolism and neurological effects of crocins and crocetin, but the mechanisms of action have not been demonstrated yet. Unknown microbial metabolites derived from crocin/crocetin and (or) changes in the fecal metabolome as a consequence of their interaction with intestinal bacteria might be involved in the neurocognitive effects.

Objectives

The main objective of this work was to explore the gut microbial metabolism of crocins and crocetin as well as changes in other known microbial metabolites as a consequence of the interaction of these saffron carotenoids with the fecal bacteria.

Methods

Fecal samples from two volunteers were incubated in an anaerobic chamber with crocins and crocetin, and samples were taken at different time points (between 0 and 5 days) after incubation. Samples were extracted with different protocols and analyzed by UPLC-ESI-QTOF and GC-MS. Targeted and untargeted metabolomic tools were used to identify as many compounds as possible.

Results

Crocins disappeared from the medium after 6 h of bacterial incubation and were transformed to crocetin by de-glycosilation. Although crocetin was more stable than the parent crocins, it started to decrease after 24/48 h of incubation in the presence of bacteria clearly indicating transformation by gut microbiota. Untargeted metabolomics allowed for the identification of a group of compounds only present in the medium after crocetin incubation. Dicarboxylic acids possibly derived from crocetin, were tentatively identified with reductions of double bonds and demethylations as the main reactions produced by intestinal bacteria. Increases in some medium- and long-chain fatty acids were also observed after incubation with crocetin. Here it was difficult to demonstrate that they came directly from the crocetin molecule since they are metabolites habitually present in the fecal metabolome and common to other metabolic routes.

In addition, samples (bacteria incubated with and without crocetin) were clustering into different groups considering those compounds tentatively identified as bile acids and fatty acids, suggesting possible changes of these compounds as a consequence of the interaction of crocetin with the intestinal bacteria.

Conclusion

This work has shown the involvement of gut microbiota in the metabolism of saffron carotenoids. The rapid transformation of crocin into crocetin and the further metabolism of crocetin to other new metabolites was confirmed. Crocetin-derived metabolites, mainly dicarboxylic acids produced after reduction and demethylation reactions were identified. Besides, our results also suggest possible changes in fatty acids and bile acids as a consequence of the interaction of crocetin with gut bacteria. Identifying all these changes at the metabolome level will be crucial to decipher the mechanisms by which the protective effects of apocarotenoids might be exerted.

P 178 THE EFFECT OF PROBIOTIC FOOD CONSUMPTION ON PHYSIOLOGICAL RESPONSES ASSOCIATED WITH REGULATION OF APPETITE: A SYSTEMATIC REVIEW

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Background:

Conditions involving appetite dysregulation such as anorexia and obesity are detrimental to quality of life whilst being a major burden to the health care system. Novel treatments, such as the use of probiotics, are of increased interest due to their potential to regulate appetite *via* the modulation of the gut microbiota. Probiotics are living microorganisms that can confer health benefits to the host upon consumption in sufficient numbers. The survival and efficacy of probiotics in the gastrointestinal tract may also be assisted with the composition of the food matrix. Despite increasing demand for probiotic foods, there is still a limited understanding of the effects of probiotics delivered in a food matrix on appetite regulation. Therefore, this systematic review aimed to investigate the effects of probiotic-containing food and beverages on the physiological responses of appetite regulation.

Methods:

Following PRISMA 2020 guidelines, literature searches using predefined terminology (MeSH) and selection of appropriate keywords for relevant studies were conducted in nine electronic databases (Scopus, Web of Science, PubMed, CINAHL, PsychInfo, CAB Abstracts, FSTA, AGRICOLA, Cochrane) with studies published in English since journal inception until March 2023. Randomised control trials involving non-pregnant and non-lactating adults, 18–65 years of age were included. Only interventions consisting of probiotics delivered in a food matrix with probiotic dosages above 10^6 CFU/mL or g and administration of four weeks or longer were included in the study. Risk of bias was performed with the Cochrane Risk-Of-Bias (RoB 2) tool for randomized control trials. (Pending PROSPERO (University of York, UK) Registration).

Results:

In total, eight studies were included in the final analysis consisting of 501 participants (M=192, F=309). Food interventions included kefir (n=1), grain-based products (n=2), and yoghurt (n=5). The length of the studies varied between 8 weeks (n=4), 10 weeks (n=2), and 12 weeks (n=2). The primary outcomes relating to physiological responses assessed were: insulin levels (n=6), leptin (n=2), adiponectin (n=1), GLP-1 and ghrelin (n=2), and secondary outcomes including body weight (n=6), body mass index (n=7), waist circumference (n=3), fat mass (n=1), body fat percentage (n=2), and appetite sensation (measured on a visual analogue scale (VAS)) ratings (n=1). A possible effect of probiotics delivered in a food matrix was found on serum insulin levels, with intervention leading to a significant decrease in three studies ($p<0.05$). Additionally, reductions were found for fat mass, body fat percentage, hunger (VAS) and desire to eat (VAS) (all $p<0.05$).

Conclusion:

The consumption of probiotic-containing food and beverages may affect insulin regulation and subsequently appetite control. However, there is insufficient evidence to suggest that such foods influence other physiological measures involved in appetite regulation assessed in this systematic literature review. Therefore, further research on the effect of food as a carrier of probiotics is required to determine its potential role in appetite regulation.

P 179 METABOLIC FATE OF DIETARY FIBRE FRACTIONS RICH IN (POLY) PHENOLS OBTAINED FROM BERRIES

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Berries are widely acknowledged for their potential health benefits due to their chemical composition. During growing, harvesting and processing of these fruits significant amounts of by-products are generated, which are typically discarded. Nonetheless, these by-products remain the chemical composition of the berries and represent a valuable source of bioactive compounds, mainly (poly)phenols (PP) and dietary fibre (DF). This study aimed to explore the use of berry by-products to isolate DF and PP-rich ingredients, and to understand how the interaction between DF and PP determine their metabolic potential. To achieve this objective, the isolated ingredients (freeze-dried berries (FDB), insoluble fibre-fraction (IFF), soluble fibre-fraction (SFF), and a PP-rich extract (PRE)) were characterised and their catabolite production were analysed after *in vitro* faecal fermentations. HPLC-DAD was used for the characterisation of both extractable (EPP) and non-extractable (NEPP) PP in the ingredients. Furthermore, *in vitro* batch fermentations were conducted using a pool of human faeces, determining the PP metabolism and microbial catabolite production throughout the fermentation period (0, 4, 8, 24, and 48 h) using UHPLC-HRMS. The results showed that FDB and IFF had the highest content of EPP, with anthocyanins as the main components. The NEPP fraction was mainly represented by ellagic acid (EA), with IFF showing the highest content. The total PP content, as the sum of EPP and NEPP, was 1.4 and 1.5-fold in IFF compared to FDB and SFF, respectively. Finally, regarding PP metabolite and microbial catabolite production, the highest amount of EA was observed at the beginning of fermentation in IFF, which gradually decreased over time. In FDB and PRE, the amount of EA also decreased during fermentation, while in SFF, it remained constant. Several urolithins were identified as degradation products of EA, with M5 and E reaching the maximum production peak at 8 h, and D and M6 were also identified. Urolithin C, M7, and A followed a similar trend with the highest production at 24 h that was maintained until 48 h. IFF had the highest production of all urolithins, followed by FDB. SFF did not yield a high production because it was not bioaccessible due to gel formation, while PRE yielded lower production due to its composition containing only extractable EA. Regarding epicatechin, the highest concentration was observed in IFF and FDB with the greatest release at 8 h. The SFF and PRE fractions showed a minority concentration with the highest production at 24 h. Within this degradation route, the PRE fraction showed the highest production of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and 5-(3›,4›-dihydroxyphenyl)-γ-hydroxyvaleric acid. In contrast, it was the IFF fraction that showed the largest production of 5-(3′-hydroxyphenyl)valeric acid, 5-(3′,4′-dihydroxyphenyl)valeric acid and 3›,4›-dihydroxyphenylacetic acid, indicating a progressive release of the parent compounds potentially bound to the DF. In conclusion, the results revealed that the DF-PP interaction influences the PP metabolism, depending on the DF attributes. Insoluble fibre, for instance, induces a gradual release of parent compounds and leads to increased catabolite production. Conversely, a greater presence of soluble fibre reduces the bioaccessibility of these compounds, potentially due to gel formation, resulting in reduced catabolite formation.

P 180 *IN VITRO* CATABOLISM AND GUT MICROBIOTA MODULATION EFFECT OF SAFFRON AND SAFFRON FLOWER (POLY)PHENOLS USING A HUMAN FERMENTATION MODEL

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Saffron is a spice widely used in the Mediterranean area, obtained from dried stigmas of *Crocus sativus*. During processing, a large number of by-products are generated, and they represent valuable products to be reintroduced into the food chain, due to their high content in bioactive compounds, such as (poly)phenols. Saffron and its floral by-products are rich in flavonols, mainly kaempferol-3-*O*-sophoroside-7-*O*-glucoside and quercetin-3-*O*-sophoroside-7-*O*-glucoside, and anthocyanins. These compounds have been recognised for their antioxidant, anti-inflammatory and vasodilator activity, but most of them are resistant to the digestion process and reach intact the colon, where they are catabolised by the gut microbiota into catabolites, which could exhibit higher bioactivity than their parent compounds. On the other side, the presence of these compounds in the colon could modulate the gut microbiota towards a healthier profile, thus possibly improving the health of the host.

The main aim of this research was to evaluate the microbial digestion of bioactives of saffron and its main by-product, and to investigate their ability to modulate the microbial profile, using a human *in vitro* fermentation model. To achieve this objective, an *in vitro* biotransformation model using faecal material from healthy donors was applied. Briefly, faecal medium (45%, v/v) and growth medium (45%, v/v) have been added to saffron samples (10%, v/v). The fermentation process was conducted in sealed vials after nitrogen flush to guarantee anaerobic conditions. Incubation was performed for 48 h at 37 °C in a shaking bath. Aliquots, collected at different time points, were analysed by high-performance liquid chromatography coupled with a mass spectrometer (uHPLC-MS/MS), to check for saffron and saffron flower catabolite production. Moreover, after DNA extraction, microbiota taxonomy at the species level was analysed, applying targeted amplicon sequencing based on internal transcribed spacer (ITS) protocol, targeting the entire spacer region between the 16S rRNA and 23 rRNA genes within the rRNA locus.

The results of this study will contribute to increase the knowledge about the functional properties of saffron and its floral by-product on the production of potentially bioactive catabolites and the modulation ability towards the gut microbiota profile.

This work is part of the “Valorisation of saffron and its floral by-products as sustainable innovative sources for the development of high added-value food products” – SAFFROMFOOD – PRIMA Program, Agro-food Value Chain, Topic 3.1- Valorising food products from traditional Mediterranean diet.

P 181 IMPACT OF (POLY)PHENOL-RICH FOODS CONSUMPTION ON GUT MICROBIOTA IN POSTMENOPAUSAL WOMEN

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The decline of estrogen levels observed in postmenopausal women can adversely impact women's health and may contribute to the development of obesity, metabolic syndrome, cancer, and cardiovascular disease, among others. One of the main regulators of circulating estrogens is the gut microbiota, through bacterial enzymes that deconjugate estrogens into the active form. However, this process can be altered by lower microbial diversity. Dietary (poly)phenols might prevent menopause-related disorders by the modulation of the microbiome, since a high proportion of dietary (poly)phenols reaches the large intestine intact that, together with other substances such as dietary fiber, can act as a substrate for the gut microbiota.

The aim of this research was to evaluate the impact of daily consumption of (poly)phenol-rich foods on microbiota composition in postmenopausal women. In order to achieve this aim, a clinical trial (NCT05255367) was carried out with 26 postmenopausal women, who were supplemented daily their usual diet with dark chocolate, green tea, and a fruit juice of berries, pomegranate and orange for two months. Feces samples were taken at the beginning of the nutritional intervention (T1) and at the end (T2), and analyzed by 16S rRNA V3-V4 amplicon sequencing to evaluate diversity and composition of the dominant microbiota. Relative abundances of specific bacteria as well as alpha-diversity indices (Chao1: richness and Shannon: diversity) were obtained. Beta-diversity was studied using principal coordinates analysis (PCoA) to visually display patterns of beta-diversity through a distance matrix containing a dissimilarity value for each pairwise sample comparison.

The results showed that the usual diet supplemented with (poly)phenol-rich foods for 2 months caused changes in the gut microbiota of postmenopausal women. Alpha-diversity indices (Chao1 and Shannon) were higher after the supplementation showing that dietary (poly)phenols were able to increase microbial richness and diversity. Beta-diversity showed that microbiota is different before and after intervention. At the genus level, the relative abundances of *Faecalibacterium, Dorea, NK4A214_group, Ralstonia,* and *Eubacterium eligens* significantly increased after the supplementation of polyphenol-rich foods in postmenopausal women.

Our findings suggest that dietary (poly)phenols and their metabolites play an important role in modulating the gut microbiota on specific bacteria and the consumption of (poly)phenol-rich foods could be considered interesting dietary strategies for the management of menopause-related disorders.

P 182 NUTRITIONAL COMPOSITION AND ANTI-DIABETIC EFFICACY OF EDIBLE INSECTS AND CORDYCEPS-CULTIVATED INSECTS

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Insects are emerging as a new alternative food due to various nutrients. Nutrients of insects are quite different depending on species. Insects are also used as substrates for Cordyceps mushrooms. Cordyceps, which originally grows on insects, is used as foods and traditional medicines. Cordycepin has been reported as the main active ingredient of *Cordyceps militaris* with diverse activities. Therefore, after cultivation of Cordyceps using six different edible insects, the components and efficacy of insects and Cordyceps-insects were analyzed. Nutritional changes after cultivation with Cordyceps were analyzed by measuring the amounts of protein, carbohydrate and fat. Nutritional composition of insects was different depending on type. The protein content in insect was increased after the cultivation with Cordyceps whereas carbohydrate content was decreased. The fat content showed differential trends for each insect. All insects showed excellent anti-diabetic efficacy by increasing glucose absorption, as there was a difference between insects. In addition, cordycepin was produced in Cordyceps-insects, which showed great differences among insects. All insects and Cordyceps-insects showed excellent anti-diabetic efficacy by increasing glucose absorption. Among six edible insects, mealworms and Cordyceps-cultivated-mealworms showed the most excellent efficacy. Our present study showed that insects and Cordyceps-cultivated-insects have not only nutritional components but also anti-diabetic effects. Therefore, they are expected to be valuable as foods and functional ingredients.

P 183 MODULATION OF THE EXPRESSION OF UMAMI, SWEET AND BITTER TASTE RECEPTORS IN WISTAR RATS BY THE INSECT PROTEIN CONSUMPTION

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Some species of insects are a sustainable source of good biological protein for humans. There are some studies regarding their bioactivity at the gastrointestinal tract (GIT), but there is no information regarding their possible interaction with the extraoral taste receptors located along the GIT. Some of these taste receptors (tas1r and tas2r) has been reported to be modulated by some protein-derived peptides and/or amino acids.

In the present work, the expression of taste receptors was analysed in a standard diet, in a diet chronically supplemented with insect protein and with or without endotoxin-induced inflammation. The study consisted of five groups of female Wistar rats: a group fed a standard diet (Control); a group fed a standard diet plus five days of i.p. administration of LPS injection (Control + LPS); groups supplemented with a daily dose of *Tenebrio molitor* flour for four weeks, with (Tenebrio + LPS) or without (Tenebrio) LPS injection; and the group supplemented with a daily dose of *Alphitobius diaperinus* (Buffalo) flour. The expression of tas1r and tas2r in the intestinal tract of these rats was analyzed and correlated with biochemical and inflammatory parameters. The results showed that insect consumption modulates the expression of some taste receptors in different sections of the gastrointestinal tract, being jejunum the most sensitive. In addition, the expression of Tas2r119 and Tas2r138 in different sections of the intestine correlated with some inflammatory parameters and intestinal permeability. The expression of umami taste receptors in the colon correlates positively with ghrelin secretion, whereas duodenal and colonic expression of tas1r and tas2r correlates negatively with circulating triglyceride levels.

In conclusion, the expression of taste receptors may be modulated by insect consumption or LPS administration, and correlates with the levels of some plasma and intestinal parameters.

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